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VIRULENCE AND IMMUNIZING CAPACITY OF SALMONELLA TYPHIMURIUM AS RELATED TO MUTATIONS IN METABOLIC REQUIREMENTS[1]

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ENVIRONMENTAL and genetic influences acting on and within pathogen and host have been shown to be important in resistance to disease. The level of mortality and morbidity of infectious typhoid in domestic fowl or mice as shown by the many years of research at Iowa State College is dependent upon the proper relations between the genetic constitutions of the pathogen and of the host. In a sense, the severity of a disease may be represented as a surface of a solid model. The genetic constitutions of the hosts are on one side of the base. At one end specified doses of the pathogen cause complete mortality to hosts of the highly susceptible constitution. In the middle, like doses induce 50% deaths. At the other end, similar doses scarcely affect the activities of the hosts (GOWEN 1948, 1952).

The genetically different typhoid pathogens are on the other side of the square. At one end, a genetic line of the bacteria is practically innocuous to any strain of the host. In the middle, the genotypes of the lines endow them with capacities to kill most of the susceptible mice, allow the survival of about 50 percent of the medium susceptible mice, and nearly all of the resistant mice. At the other end, the virulence of the pathogen line is so great that some mice of even the resistant strain die when inoculated with the standard dose of organisms.

Over the surface of the square the different genetic constitutions of host and pathogen fit together in such a manner as to give all types of disease reactions. This key to lock mechanism suggests the next steps if disease is to be understood. Information is needed on what the genes do to modify the different lines of the pathogen and thus induce the different disease reactions. A growing body of evidence indicates that a gene causes its primary reaction by contributing to the synthesis of a required chemical at some particular step in its formation. For a pathogen some of these steps may be significant to the organism's invasive ability as in the release of toxic metabolites, endotoxins, and in competition for host metabolites, etc.

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To test these questions and to search for lines of pathogens which may be utilized in further virulence studies, mutants unable to synthesize certain essential growth elements were isolated from three pure cultures of *Salmonella typhimurium* over a period 1946 to 1950. The first culture, 519, originally came from a human food poisoning case in New York. The second and third cultures, 533 and 533-11C, were transfers from our line 11C, under study in our laboratory for some 25 years. The mutants were obtained as isolates from X-ray or ultraviolet treated cultures. Mutants are specified by number and the growth substance they have come to require at the time of mutation. These isolations were made at Amherst College by H. H. PLOUGH and HELEN MILLER. The techniques of initiation and separation of these mutants may be found in the literature cited (PLOUGH, YOUNG and GRIMM 1950; PLOUGH, MILLER and BERRY 1951).

The studies of natural resistance and acquired immunity were conducted at Iowa State College by J. W. GOWEN and J. STADLER, with the assistance of DOUGLAS GRAHN and PATRICIA BANACH, during 1948 to 1951.

TABLE 1

Differences between experiments within strains of mice. Tests with S. typhimurium line 11C.

Strain of mice	Number tested	Percent survived	Between experiments within strains		
			χ^2	d.f.	P
Balb/Gw = Ba in text	120	0	0	4	1.0
LGW = L in text	33	33	2.4	3	.5
Z	35	77	8.0	3	.05
S	29	100	0	2	1.0

Sixty-seven independent *S. typhimurium* mutants and their four parent lines were randomly selected for this study. Twelve mutants came from culture 519, 30 from 533 and 25 from 533-11C. These mutants were tested for their virulence on four of our inbred strains of mice. 200,000 organisms per mouse were injected intraperitoneally in each mouse tested. The results of these tests measure the virulence of the mutant bacterial line.

After recovery from the experimental typhoid infection, mice acquire some active immunity. The extent of this immunity was assayed by inoculating a challenge dose of 50 million virulent 11C organisms. The results of this test measure the power of the mutant bacterial line to stimulate active immunity in the host. Certain factors, significant to the adequacy of the tests, as strain and sex of host, average virulence or immunizing power of the mutants, repeatability of tests, etc., will be examined before considering the mutants individually.

Repeatability of virulence tests

Five separate experiments were conducted. Four inbred strains of mice served as the test hosts. Mouse typhoid culture line 11C was used as a control in each of the experiments. Table 1 shows how repeatable these tests were.

TABLE 2

Sex differences in resistance—First test. Strain Ba.

Experiment	Total mice tested	Percent survived		χ^2	P
		Males	Females		
I & II	475	85.6	90.8	2.9	.10
III	463	66.1	60.1	1.9	.15
IV	472	23.1	21.9	.1	.6
V	1092	80.4	83.1	1.6	.2
Total	2502	68.2	68.5	.7	.4

The P values for the different strains indicate that the different experiments furnish similar information.

The percent survival for the different mouse strains shows the host effects range from the highly susceptible Ba mice to the highly resistant S mice. The L and Z strains were somewhat more resistant than expected from the large accumulated body of data available from past work.

The resistance of the S mice is such that they will withstand attacks of almost any line of *Salmonella typhimurium*. This 100 percent survival does not allow differentiation of virulence in the various mutants. Similar considerations apply to the Z mice and to a lesser extent to the L mice. The Ba mice contribute the greatest amount of information so these data are chosen for presentation.

The data of the different experiments within strains of mice were comparable so they were combined for further analysis.

Sex effects on resistance

In general the individual tests of the different mutants were balanced as to sex of the host. Table 2 gives the survival value for the males and females in each experiment together with the χ^2 tests for the homogeneity of the results.

The data on Ba mice of table 2 show that the males and females react in like manner to their initial contact with the mouse typhoid organisms, probabilities being such as easily obtained through chance difference.

Table 3 gives similar data on the challenge tests for active immunity.

TABLE 3

Sex differences in resistance—Challenge test. Strain Ba.

Experiment	Total mice tested	Percent survived		χ^2	P
		Males	Females		
I & II	394	58.9	65.4	1.8	.19
III	265	64.8	54.0	3.2	.07
IV	100	70.4	58.7	1.5	.22
V	804	45.0	40.6	1.6	.20
Total	1563	53.4	50.5	1.2	.27

These data indicate that the sexes become actively immunized to the typhoid organisms of the different lines to a like extent. The data for the sexes are combined in the rest of the study.

Comparison of host strain survivals for all mouse typhoid lines

The inbred mouse strains utilized in these tests exhibit established differences in resistance. The differences as tested by a challenge dose with the virulent 11C parent culture were shown in table 1. The differences when the mice are challenged by the mutant *S. typhimurium* lines are shown in table 4.

The host strain differences in resistance are evident in table 4 where all of the different mutant strains are grouped together. The survivals of all strains on first contact with the disease are greater than those observed when these mice are exposed to the parent strain *S. typhimurium* 11C. As would be expected these differences in susceptibility become more evident as the tests progress from the resistant toward the more susceptible hosts. On the average, mutation of a virulent bacterium tends to reduce the virulence of the parent bacterial lines.

TABLE 4

Survival of mice where the mutant S. typhimurium lines initiated the disease.

Strain	First test			Challenge test		
	Number tested	Number survived	Percent survived	Number tested	Number survived	Percent survived
Ba	2262	1649	73	1563	803	51
L	807	653	81	553	373	67
Z	667	580	87	573	482	84
S	497	488	98	440	412	94

The progressively increasing proportion of survivals from the susceptible Ba mice to the resistant S show that the acquired immunities as well as the natural resistances are under genetic control of the host. Our earlier immunization experiments utilizing line 11C and two of its derivatives led to like conclusions (GOWEN 1937–1947).

The immunity acquired by the mice from the mutant *S. typhimurium* lines, table 4, is generally less than the immunity acquired when the parent culture 533-11C offers the initial challenge (100 percent survival for all hosts in these experiments). Mutation, on the average, has lowered the immunizing ability of the mutant line.

The general reduction in virulence of the mutants tends to reduce the differences in the outcome of the tests for virulence as the mouse strains employed in the tests are taken from stocks of higher natural resistance. In other words the data from the experiments on the Ba mice are most significant for separating the mutant effects on virulence and immunizing against mouse typhoid. The experiments employing the L, Z and S are respectively less and less significant. For this reason most of the subsequent analyses will deal with the

TABLE 5

Relation between natural resistance or acquired immunity of Ba and L mice tested with different S. typhimurium mutants.

Constants	Natural resistance	Acquired immunity
Mean resistance of Ba, % survival	65	65
Mean resistance of L, % survival	91	56
Correlation coefficient between Ba and L resistance	0.61**	0.88**

*Shows significance in the 0.05–0.01 probability range.
**Beyond 0.01 range for this and subsequent tables.

results of experiments in which the Ba strain of mice was employed and omit those for the other host strains.

Before leaving this point, however, data are presented below to show that, when tested in the same experiments, the results secured with Ba mice have a high correlation with results on the L mice in both the response to first contact with the disease and in the acquired immunity tests.

The different strains of mice are known to have certain elements in common as well as certain differences which determine their respective resistance to *S. typhimurium*. The correlations of table 5 measure these effects. The Ba and L mice show quite similar reactions even though the L mice are distinctly more resistant than the Ba strain. It appears probable that if the dosages of test organisms and other conditions were adjusted properly, the Z and S strains of mice would show common pathways of resistance to the different *S. typhimurium* mutants comparable with those observed for the Ba and L mice as well as unlike mechanism of resistance having importance to the given strain. As the experiments were conducted, the spread in survival rates is greater for the Ba mice than for those of any of the other strains. The Ba mice consequently give a clearer differentiation for separating the effects of mutations on virulence and acquired immunity. This strain is used for the subsequent evaluations of the mutants.

TABLE 6

Variance of bacterial mutants in virulence—First tests. Ba mice. Bacterial lines.

Source of variance	d.f.		Sum of squares		M.S.	
	All data	Only those with repeat tests	All data	Only those with repeat tests	All data	Only those with repeat tests
Among bacterial lines	66	36	313.3	98.1	4.75**	2.72**
Within bacterial lines among tests	42	42	29.5	29.5	.69**	.70**
Within bacterial lines and tests	2152	1106	104.1	72.0	.05	.07
Total	2262	1184	446.9	199.6		

Stability of virulence and immunizing properties of the S. typhimurium mutants

The metabolite mutants were repeatedly tested within the different experiments. These experiments give a measure of the consistency with which the different mutants maintain their virulence. The data are shown in table 6.

The variance analysis of the binomial data, table 6, shows that the *S. typhimurium* mutants are different from each other in their virulence.

The experiments themselves were shown to be homogeneous when tested with the 11C culture, P = 0.5 to 1.0 for the four host strains, data not shown. The test difference within bacterial lines shown may be attributed to some balancing interaction between local conditions within the experiment.

A similar analysis for the stability of the immunizing properties of the mutants is shown in table 7. The different *S. typhimurium* mutants are shown to be significantly differentiated from each other in their abilities to stimulate acquired resistance in the host. These mutants display some differences in their abilities to immunize in different experiments.

TABLE 7

Variance of bacterial mutants in virulence—Challenge tests. Ba mice. Bacterial lines.

Source of variance	d.f.		Sum of squares		M.S.	
	All data	Only those with repeat tests	All data	Only those with repeat tests	All data	Only those with repeat tests
Among bacterial lines	54	35	218.9	101.1	4.05**	3.0**
Within bacterial lines among tests	40	40	26.3	25.9	.65**	.65**
Within bacterial lines and tests	1411	784	131.2	87.0	.09	.11
Total	1505	857	376.4	214.1		

Effect of metabolite requirement on pathogenic properties of S. typhimurium mutants

The bacterial mutant lines were of several types. Thirty-nine of the 67 mutants require but a single known metabolite for growth. Fourteen were shown to require one of two different metabolites. Of this number six lines were composed of two different and separable bacterial types, one requiring one metabolite and the second type requiring the other metabolite. Presumably these six lines arose in the original culture as separately mutated bacteria which stuck together on transfer, or as two mutations in different nuclei of a multinucleate bacterium. In the latter case the two nuclei would subsequently separate by division and become incorporated in independent organisms as a consequence of later cell division. The same possibility may account for the so-called delayed mutation effect noted in other bacterial work. The eight true

alternate types may be accounted for as mutants affecting a single chain of chemical syntheses that results in the formation of a common end product.

Four mutant lines showed alternative requirements for three substances. These four lines arose like those above for the six lines having alternatives due to mixture of distinct bacterial types. Three mutant lines were of a single type requiring two metabolites. These lines were formed by irradiating mutants a second time and then separating out the second mutants to give lines having the double requirements. Two mutant lines had three metabolite requirements made again by successive irradiations and separations of the three mutant forms. One mutant line had four requirements. Four mutant lines required more than four known metabolites. The metabolites considered were adenine, arginine, cystathionine, cysteine, glutamic acid, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and antigens. The different mutants distributed for virulence and immunizing ability are shown on the scatter diagram together with the metabolite they now require.

The mutants are all located in the left hand diagonal half of figure 1. There is a high positive curvilinear correlation between the virulence of the mutant in its capacity to initiate the disease and the active immunity generated in the host due to its reaction with the mutant. Examination of the data shows that if the capacity of a mutant to cause immunity in the host is considered as increasing as the logarithm of its capacity to cause death and morbidity then a simple linear relation will exist. This relation is described by the equation

$$\textit{acquired resistance} = a + b\,(\log_{10} \textit{virulence on first contact})$$

The acquired resistance is measured as the percentage of mice which survive the challenge dose of 11C *S. typhimurium* mutant, the a may be looked on as the average resistance to avirulent mutants at first contact with the 200,000 dose, b is the regression coefficient. The virulence on first contact is measured by the percentage dead after inoculation (plus 1 to avoid zero values) with a dose of 200,000 mutant *S. typhimurium*. The observed equation is

$$\text{acquired resistance} = 34.6 + 33.8\,[\log_{10}\%\ (\text{dead} + 1)]$$

The graph portraying the trend of acquired resistance with successive degrees of resistance is shown as the curved line on the chart. The correlation of natural and acquired resistance is 0.69 when the data are considered in this manner. Measured on the scale of variance controlled, there is a gain in estimating acquired immunity of 37 percent when the estimate is made from evidence on natural virulence of the organism.

Comparison of the average trend line with the original observations shows that most of the trend variation in the data is removed by this line.

It is known that *S. typhimurium* organizes an endotoxin. The results of figure 1 could be interpreted as indicating that the quantity of endotoxin produced by the bacterial line was governed by genes in many loci. The quantity of endotoxin could create the conditions necessary for growth in the host. Mutation could affect the endotoxin production in many ways. The virulence

tests would indicate how the endotoxin was increased or decreased by mutation. The acquired resistance tests would indicate the capacity of the quantity of endotoxin generated by the bacteria to induce the immunity in the host. Endotoxin in small amounts would raise the acquired resistance a small amount. Increasingly greater amounts would increase the acquired resistance by the logarithm of the amount rather than as a directly additive increment. Endotoxin dosage could be considered as measured by the virulence tests. Immunizing effects can be titrated by the acquired resistance observed after the tests. The *in vivo* virulence-immunizing reactions would then fall in the pattern of a number of toxicological substances in which the endotoxin was the agent subject to genetic modification as described by the equations presented above.

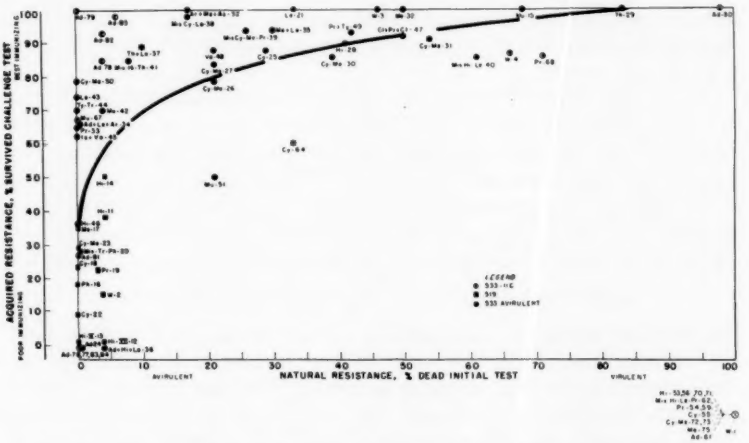

FIGURE 1.—Distribution of bacterial mutants tested for virulence and for immunizing capacity.

But the explanation is seemingly not that simple. HILL, HATSWELL and TOPLEY (1940) compared the genetic resistance to an extracted endotoxin of *S. typhimurium* as selected out of a group of mice over a number of generations with resistance to a challenge dose of intact organism. They found no correlation between the two. This result would seem to indicate that the disease syndrome has, as independent variables in the severity of the disease, capacity of the organism to grow in the host and the endotoxin produced by the pathogen. In the face of this evidence, all of the observed relations of the data in figure 1 cannot be attributed to the endotoxin effect.

Considered individually there are mutants which hold the virulence of their original parent lines and those which do not. In the right hand lower corner of the chart are 12 mutants from 533-11C. These mutants retained the virulence of the 11C (indicated by W_1) parent of 533-11C to the extent that these

mutants killed all of the mice in which they were introduced. The mutants included four which require histidine, two require proline, two cysteine or methionine, one each requires cysteine, methionine, adenine or a mixture of histidine, leucine and proline. Similarly adenine-80 and proline-68 were both more virulent than their parent 533-11C (indicated by W-4).

Several mutants lost virulence. Adenines-76, 77, 79, 81, 83, 84 and multiple-67 lost all virulence. Adenines-78, 82 and 85 lost nearly all virulence. Cysteine-64 lost half of the parent virulence following mutation. 533-11C itself has lost virulence when compared with its 11C parent, 67 percent deaths as compared to 100 percent.

Parent 533, W-3, an earlier direct transfer of 11C, lost more virulence than 533-11C. Its average virulence on test was 45 percent deaths. The drop in virulence after transfer from 11C is disturbing. In the more than 25 years that 11C culture series has been kept in this laboratory no change in virulence has been noted in the course of many tests yearly. 11C is known to mutate (ZELLE 1942) to forms showing decreased or increased virulence when separated into pure lines, but as it is kept by us has not itself reflected any changes by differences in virulence or general morphological or cultural characteristics between the successive transfers. The 533 transfers were shipped from Ames, Iowa to Amherst, Massachusetts. They were kept as stab cultures on brain heart infusion agar.

The differences in treatment are the only known selective agents which might favor particular spontaneous mutants of a greater growth capacity on culture media and possibly lower virulence. Experimental check is required to determine if this tendency to lose virulence may be accounted for by these cultural conditions.

533 was one source of mutants having different metabolite requirements. Threonine-29 was quite virulent. Threonine and leucine requiring mutant-37 had little virulence. Adenine-24 from the same source had little virulence. Other metabolite requiring mutants had virulence tests of all intermediate types.

Parent culture 519, W-2, although isolated from a human case had little virulence for our highly susceptible Ba mice. In general mutants from this parent type were likewise quite avirulent. One culture, leucine-21, alone of all the rest, increased markedly in virulence. This culture showed an even more pronounced change away from its parent's characteristics. It had the capacity to stimulate acquired immunity reactions in the host; animals which survived the initial infection with this mutant were 100 percent resistant on retest whereas only 15 percent of those exposed to the parent 519 were able to survive the challenge.

The immunizing abilities of the different metabolite mutants show the same types of variation that are observed in the initial tests for virulence. Mutants for adenine requiring 76, 77, 83, 89 of 533-11C are avirulent and poor immunizing. Mutant 81 requiring adenine and avirulent stimulates the host immune system to some degree. Adenine requiring mutants 78, 79, 82 and 85

are avirulent but have the best immunizing ability. Mutant adenine-80 requiring is virulent and immunizes solidly. Mutant-61 is so virulent that no mice survive for test of its immunizing ability. Comparisons for the other mutants and their parents bring out similar variations within other metabolite requirements.

Relation of mutant virulence to that of parent line

The data show that mutation to a given requirement may be accompanied by all degrees of change in virulence. The average change when the parent line is virulent is some reduction in virulence and a lowering of immunizing ability. The data in figure 2 bring out these facts. In figure 2 the mutants of 519 are shown as solid squares, ■, those of 533 as solid circles, ●, and those of

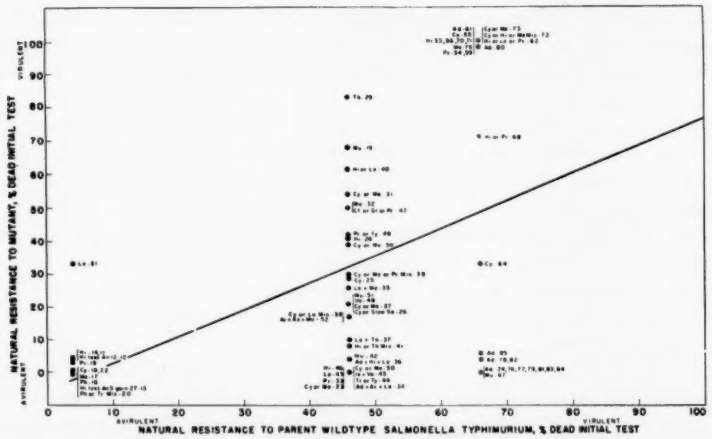

FIGURE 2.—Relation between the virulence of the parent line of *S. typhimurium* and the virulence of the mutants.

533-11C as the open circle with the cross, ⊗. The mutant's virulence plotted against that of the parent line shows that a correlation exists between the virulence of the parent and that of the mutants which occur in this line. The product moment correlation for these data is 0.45. The average trend is apparently satisfactorily represented by the linear regression equation drawn on the chart.

$$\text{Percent dead of mutant} = 0.82\,(\%\text{ dead of parent line}) - 5.9$$

The surface contour of the parent-mutant virulence is peculiar in that the sections take J- or U-shaped forms. For 519 the mutant virulences show a clear-cut J-shape frequency distribution. Most mutants are without virulence. Only one, leucine-21, has a greater virulence than the parent. 533 mutants show the same type of effect but the J is less extreme. Most mutants are toward no virulence. Only seven mutants have greater virulence than the

parent. The mutants of 533-11C show a U-shaped frequency distribution. They are either completely virulent or are avirulent. Only two mutants out of the total number, 26, display sure intermediate virulence. The parent type had the highest virulence of those under test. Several types of nutritional mutants are noted in the virulent group. The avirulent mutants were largely adenine mutants, but this may have no particular meaning since there are also adenine mutants at the high virulence end of the distribution. The character of the surface contours of figure 2 makes it clear that no particular emphasis is to be put on the exact value of the correlation.

The data indicate that in general the observed effect of mutation on virulence is directional in that, even though the most susceptible mouse strain known to us was the test animal, the avirulent strains, if they are to survive,

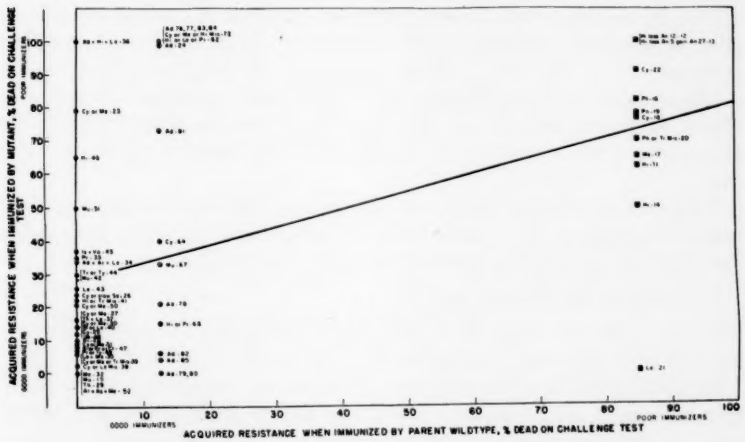

FIGURE 3.—Resistance acquired by mice exposed to the various mutants plotted against that of their parent lines as measured by percent dead on challenge by *S. typhimurium* 11C.

in a sense have only one direction in which the virulences may change, they must increase. Similarly the virulent parents have more opportunity to decrease than to increase in virulence. Increases occur but in most instances the direction of change is toward lesser virulence. These facts conform to those brought out for *Phytomonas stewartii* some years ago by LINCOLN and GOWEN (1942) in their study of the same problem.

Relation between mutant immunizing power and that of its parent line when challenged by virulent 11C

The immunizing powers of the different mutants are plotted against those of their parent lines in figure 3. The significance of the symbols is the same as with the previous charts. As an immunizer 533 proved better than 533-11C. Both were distinctly better than 519. Comparison of figures 2 and 3 shows

that 533 and 533-11C have changed their order. In virulence 533-11C had greater virulence than 533 whereas for immunizing ability 533 stimulated greater immunity than 533-11C. The differences between these lines are not great in either case but they do suggest that lethality and immunizing power are not completely correlated. This fact is further substantiated by a comparison of the individual mutants.

The mutants of 533 range in immunizing power from good to poor or none. The distribution of the mutants is again J-shaped. The larger number of mutants fall in the good immunizer class as did the parent line. A few of the mutants, notably the multiple adenine plus histidine plus leucine-36 and the alternate cysteine or methionine-23, have had their particular metabolite mutations accompanied by sudden losses in immunizing power. These mutants show that the particular changes may effect immunizing power in several ways.

533-11C mutants show similar variations although in general the adverse effect of metabolite mutation on its subsequent immunizing ability is more extreme.

The mutants of the poor immunizer line 519 show a range in immunizing ability. In general immunizing ability increases when mutation occurs in this strain—one type, leucine-21, is the best and most striking example of this change.

There is a correlation between the immunizing ability of the parent line and that of the mutants. The average trend for the mutants is given by the straight line. The correlation value 0.49 and the equation of the line

$$\%\ \text{dead on challenge of mutant} = 0.53\,(\%\ \text{dead on challenge of parent line}) - 28.2$$

are subject to the same comments as those discussed for figure 2.

Metabolite mutation generally does not affect the immunizing power of the bacteria greatly, but on occasion the change can be accompanied by a change from a good immunizer to a poor or vice versa.

Average virulence and immunizing capacity of the different metabolite mutants

The average virulence and immunizing capacity of the different metabolite mutants is shown in the following scatter diagram, figure 4.

The distribution of the metabolite requiring mutants again brings out the correlation between virulence and active immunizing ability.

A general characteristic of the mutants is a regression from the parent type of virulence or of immunizing ability.

Figure 4 shows that the average virulence of the different metabolite mutants is less than that of the parent types, W-1 and W-4. Only cystathionine and proline mutants had greater virulence than parent type 3, the other 14 metabolite mutants displayed less virulence. The *S. typhimurium* parent isolated from a human case was exceeded in virulence and in immunizing capacity by most of the mutants.

The cystathionine, cysteine, histidine, methionine and proline mutants retained medium virulence. The phenylalanine, tryptophan, isoleucine, arginine and valine mutants were nearly avirulent. These mutants differed in their immunizing powers, however. The arginine, valine and isoleucine mutants had nearly as good immunizing power as the more virulent cystathionine, methionine, cysteine or proline mutants, whereas the phenylalanine mutants were poor immunizers for the 11C challenge organism.

Mutants with different chemical requirements have average differences in their pathogenic reactions but as with the individual mutants a certain amount of randomness in the effect of the mutation on virulence and immunizing capacity appears evident.

Table 8 presents an analysis of the virulence reactions.

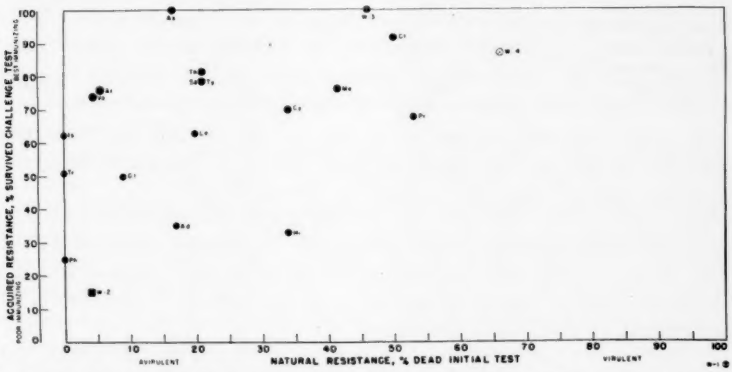

FIGURE 4.—Average virulence and immunizing capacity of the different *S. typhimurium* mutants and parental types.

There were 17 different metabolite requirements for the mutants in these data. Some mutants required two or more of the metabolites for growth. For the analysis, these mutants were considered as separate requirements. The approximations are minor with little effect on the biological interpretations to be drawn from the data.

In this method of analysis the interaction and joint effects of two or more mutations affecting different requirements are not adequately separated. These joint effects must be small. Analysis of that portion of the data in which each mutant has but one known change in its metabolite requirements leads to mean squares which are directly comparable with those of the whole data. The biological conclusions to be drawn from the sets of data are the same. The distinction between the bacterial mutant lines which have but one mutation as against those which have more may be more apparent than real. Only a limited number of metabolites out of the possible number required by the mutants of *S. typhimurium* are or can be tried out. The number of mutants observed is a function of the techniques for separation as well as of the rate of mutation. As a

TABLE 8

Variance of bacterial mutants in virulence—First test. Ba mice. Metabolites.

Source of variance	d.f.	Sum of squares	M.S.
	Mutants with single known requirement		
Among metabolite requirements	8	31	3.90
Within metabolite among lines	30	204	6.81**
Within metabolites and lines	1361	47	0.03
	All mutants		
Among metabolite requirements	16	51	3.21
Within metabolite among lines	77	392	5.08**
Within metabolites and lines	3084	188	0.06

consequence, lines where a single mutation is observed may also have other undetected mutations. The designation one, two, or more mutations is thus arbitrary, making a more complete analysis unwarranted.

Table 8 shows that the within metabolites and lines variation is small. The among metabolite and the within line requiring a metabolite variances are both large beside this standard. The metabolite mutations have changed the virulence characteristics both as to separate mutations for a given metabolite and as to mutations to different metabolites.

Comparing the between metabolites mean square with the within lines requiring a metabolite mean square shows more variation in virulence of different mutations to the same chemical requirement between mutants to differ-

TABLE 9

Variance of bacterial mutants in virulence—Challenge test. Ba mice.

Source of variance	d.f.	Sum of squares	M.S.
	Mutants with single known requirement		
Among metabolite requirements	8	18	2.20
Within metabolite among lines	22	115	5.25*
Within metabolites and lines	860	78	0.09*
	All mutants		
Among metabolite requirements	16	83	5.21
Within metabolite among lines	60	251	4.19**
Within metabolites and lines	2071	198	0.10

ent chemical requirements. This indicates that the particular chemical changes observed in the mutants may not be the primary cause in the variations in virulence observed. Rather it appears that more or less random changes in the organism occurring concurrently with mutation are the primary causes of the changes in virulence. These changes could be mutations of other genes arising simultaneously with the mutation of that for the particular metabolite, or alternatively the genes controlling a given metabolite requirement are found at several loci and these genes have multiple and different effects, the mutation of any one of which would give the metabolite requirement but a virulence different from that of another gene.

The analysis for the acquired immunity effects is presented in table 9.

The property of stimulating the host to generate active immunity shows a variation similar to that of virulence. Both the among metabolite and the within metabolite variations are highly significant. These variations are about equal. The immunizing abilities of the mutants requiring different chemicals for growth differ and differ from those of their parent strains. This suggests that immunizing ability may be affected by the particular metabolite requirement.

The fact that the different mutants for the same chemical requirement show variations like those between the different chemical requirements makes it evident that the physiological change in the chemical requirements is not the only cause of the alteration in the property of inducing active immunization. Genetically the mechanism required to explain the observed result would appear to be some combination of the following hypotheses: that the agent, X-rays or ultraviolet, causes mutations in genes governing the formation of the substances necessary for immunization simultaneously with those governing the synthesis of the metabolites under consideration or that there be several genes for the metabolite under study and these genes have as a second attribute different effects on the synthesis of the immunizing substances. At present it has not been possible to separate these hypotheses.

DISCUSSION

The variations observed in the virulence or immunizing abilities of the different mutant strains of *S. typhimurium* tested showed a correlation between the genotypes of the lines involved and their capacities to cause moderate or extensive mortality. The mutants were distinguished by their inability to reproduce without the addition to their diets of certain metabolites not required by their parent wild types. These changes in growth requirements would be expected to affect the adaptabilities of the organisms to reproduce within a host whose body fluids may be a complete medium. The different requirements of the pathogens compete with those of the host cells in new ways. Similarly the synthesis of any endotoxic substances by the pathogens, normally generated to further penetration into the host tissues, could be altered. New substances or substances in increased amounts could be formed by the mutants. The release of these materials into the host system could lead to

changes in the pathogenic pattern. A gene responsible for alterations in the normal metabolic sequence appears to cut the successive reactions at some particular step. The end products of the incomplete synthesis could pile up. Some of these products are known to be toxic. Their presence in the host system could contribute to the severity of the disease. All of these effects and others not mentioned could be expected as a consequence of a mutation in the pathogen.

Among the results there could be some due to the direct effects of the mutations on the various component parts of the syndrome which give *S. typhimurium* its ability to cause typhoid in mice: This disease property is rather highly specific. It would appear to represent some chance combination of genes which was preserved by and improved upon in successive generations of reproduction by selection of the better gene combinations and the inclusion of any of the more favorable mutations which might have occurred. Witnessing an epidemic carries the conviction that the hereditary combination of genes in the pathogen involved is efficient. It seems unlikely that random changes of the nature of mutations could be made in the gene complex involved and often improve the disease-producing machine of the highly virulent organisms. Our results agree with this expectation. The mortalities and morbidities of the mutants of a highly virulent strain are generally less than the parent. These reductions in virulence are probably explicit for the given mutant, but are not necessarily due to the criterion on which the mutant was separated from the rest of the group. The twelve adenine mutants from 533-11C tested on fifty or more mice each are illustrative of what was observed for other mutants. Seven of the adenine mutants caused no deaths, two had 4%, one had 6%, one had 98%, and one had no survivors. Each of these mutants was separated on the basis of its effect on adenine. The range in virulence effects shows that the adenine requirement is not itself the primary cause of virulence. Rather it is some change accompanying the shift in the adenine requirement.

Where the parent culture is avirulent, any observed changes in virulence will of necessity be toward higher mortality. Our parent type 519 was nearly avirulent. Of the eleven mutants only one, that for leucine-21, showed a moderate increase in virulence. The mutations as they are observed are directional in that the mutants tend to be less virulent when their parent is virulent and more virulent when the parent is avirulent. The particular change in observed virulence is not necessarily related directly to the metabolic change by which the mutated gene was detected.

BACON, BURROWS and YATES (1950) have made a study similar to ours on the virulence of biochemical mutants in *Bacterium typhosum* when introduced into commercial mice. The mice were treated in groups. One received 50 million organisms. The other mice were divided into groups injected with a range of doses to cover the ALD dose estimate for probit analysis. Their results agree in many respects with our own data. The majority of their mutants did not change in virulence. In their minor test on 10 mice the different mutants to the same requirement varied considerably in their virulence. This is in line

with the conclusion drawn above that the particular character on which the mutant was separated is generally not the primary cause of the virulence change. Rather it is the place in the chain of syntheses that the gene affects the organism or the accompanying mutations which leads to the observed changes in virulence. Aspartic acid requiring mutants and purine or purine + aneurin mutants are consistently mutants of lowest virulence in the BACON et al. (1950) experiments. In our data there is but one aspartic acid requiring mutant and that mutant also requires arginine and methionine. This mutant showed a decrease in virulence of 66 percent. The adenine mutants in our data retain the parent virulence or show decreases in virulence depending on the mutant. As within our own data so with the comparison of our data with those of BACON, BURROWS and YATES (1950), the mutant characters used in identifying the mutant are correlated with rather than causative of the changes in virulence observed.

Natural mutations toward lower or higher virulence can and do occur as demonstrated in the work of ZELLE (1942) on *S. typhimurium* in our mice; GOWEN, STADLER and BELL (1937–1947) on *S. gallinarum* in fowl; and WELLHAUSEN (1937) and LINCOLN and GOWEN (1942) in *Phytomonas stewartii* in maize. Mutation in virulence following X-radiation have been quantitatively estimated in *Phytomonas stewartii* by LINCOLN and GOWEN (1942). These results are concordant with those in this study in that they show that the capacity to mutate toward or away from increased virulence is observed. The frequencies of the changes depend on the parent type virulence and the stability of the organism. Immunization experiments by GOWEN (1945) have shown that the capacity to stimulate active immunity is likewise affected by the mutations.

In certain of these mutations colony morphology or other characteristics have been seen to vary with the mutations in virulence. These changes are again correlative changes rather than causative over the whole range of effects.

SUMMARY

Sixty-seven metabolite mutants of spontaneous, X-ray or ultraviolet induced origin are tested and analyzed for their virulence and capacity to stimulate active immunity in mice.

The mutants were from three parent stocks; 519 derived from a human case of *S. typhimurium* infection and of low virulence to mice, two derivatives of our strain 11C pathogenic to mice in proportion to the particular host genotypes in which these organisms are introduced.

Four inbred and selected strains of mice were used in these experiments. These mice behaved consistently both within the strains and among the experiments and sexes.

The data presented are largely for the most susceptible mouse strain as the data from them are most informative. The mortalities or active immunizing abilities as tested in the different strains are correlated but the mean survivals change with the strains.

The different mutant bacterial lines showed significant differences in their virulence and in their immunizing capacity. These lines have greatly extended the known mutative changes in pathogenicity and are furnishing significant material for the further analyses of this problem.

A curvilinear correlation, accounting for some 37 percent of the observed variation in active immunizing power when the virulence is known, is found between the virulence of the mutant and its power to stimulate active immunity.

The virulence of the mutant is related to the virulence of its parent. A parent of high virulence tends to have mutants of lesser virulence, a parent which is avirulent tends to have mutants of greater virulence than the parent.

Similar reactions are noted for the capacity of the mutants to stimulate active immunity. Parents having poor immunizing powers tend to have mutants which are better, but not much better, immunizers. Similarly the parent having good immunizing powers tends to have mutants with somewhat less good active immunizing stimulating capacity.

The virulence and immunizing capacity of the different nutrient requiring mutants differ more than would be expected on random sampling. However, the variation within the metabolic mutants of like requirement is greater than either that among metabolites or within metabolites and lines. These differences are interpreted as indicating that the particular chemical changes observed in the mutants may not be the primary causes of the variations observed in either virulence or immunizing capacity. The observed variations could be due to mutations of other genes arising simultaneously with the particular metabolite mutant or that the genes controlling a given metabolite requirement are found at several loci and these genes have multiple or different effects—the variation of any one of which would give the metabolite requirement but a virulence or immunity different from that of another gene.

The mutation process, which led to a line having a phenotypically different metabolic requirement from that of its parent as studied herein, had no consistent influence on either virulence or active immunizing ability of the new bacterial line.

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A GENETIC ANALYSIS OF THE MATE-KILLER TRAIT IN PARAMECIUM AURELIA, VARIETY 8[1]

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IN striking contrast to previous experience, it has been found that when certain paramecia called "mate-killers" (MK) conjugate with others called "sensitives" (S) only one of the two exconjugants of each pair will produce a fully viable clone (SIEGEL 1953). The nonviable exconjugant will usually die before it can form daughter cells. The parental mate-killer conjugant always produces the viable clone whereas the sensitive conjugant is regularly the nonviable clone. The discovery of mate-killing posed questions of genetic interest some of which are answered in this communication.

Mate-killers have been discovered only among stocks belonging to variety 8 of *Paramecium aurelia*. Of the 11 known stocks of this variety, three are mate-killers and eight are sensitives (LEVINE 1953). The experiments to be described were primarily designed to discover the genetic basis for the mate-killer trait in stock 138. They also answer the question of the occurrence of normal reciprocal fertilization among pairs which manifest mate-killing. Some new facts concerning the mechanism of mate-killing, dealt with in another paper (SIEGEL 1953), are also reported. Finally, the possible evolutionary significance of the mate-killer trait is discussed.

MATERIALS AND METHODS

The materials and general methods used in the present study have been described by SONNEBORN (1950) and SIEGEL (1953). The stocks used included the sensitive, 137 and the mate-killer, 138 (SIEGEL 1953). Stock 151, a sensitive collected from Bok Tower, Fla., was also used.

As previously described (SIEGEL 1953), pairs can usually be accurately scored as to mate-killing on the basis of the viability of the two exconjugant clones two days after their origin. At this time the clone from the mate-killer parent will consist of the progeny from five fissions while the clone from the sensitive parent has not divided and is dead or will soon die.

Crosses between diverse homozygotes were made according to the methods of Sonneborn (1950). Viable F_1 clones to be used for further crosses were allowed to expand to 10 or 12 fissions, starved, and then crossed.

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Most of the F_2 clones were obtained from crosses of $F_1 \times F_1$; others were obtained by autogamy induced in F_1 clones by the serial daily isolation method (SONNEBORN 1950). Crosses between F_1 and parental stocks yielded backcross (referred to as " BC ") generations. The F_2 and BC progeny were cultured according to the standard methods previously described for mate-killers and were tested for phenotype between the 12th and 18th fissions. At this time, some contained animals which had already, but recently, passed through autogamy. Thus some heterozygous F_2 or BC clones may have included some diverse homozygotes together with preautogamous animals. Despite this fact, as will be seen later, the phenotype of the heterozygotes was revealed by appropriate tests. Of course, cultures started from homozygous F_2 and BC clones could not result in recombination at autogamy.

Cultures can be classified as mate-killers or sensitives on the basis of results of matings with cultures of known phenotype. If a cross to a mate-killer yields pairs showing mate-killing, then the culture is a sensitive; if no death follows such a cross, then it is a mate-killer. If the culture to be identified is crossed to a known sensitive and mate-killing is observed, it is a mate-killer; if the pairs are fully viable, the culture is a sensitive. While the principle of classification is simple and straightforward, in practice certain difficulties are encountered.

First, a breeding analysis cannot serve to classify certain atypical clones containing cells which may die due to causes other than mate-killing. Such cultures are said to show " reduced viability." Other cultures are difficult to cross because they contain a mixture of animals of mating types XV and XVI. These cultures are recorded as " selfers " and remain unclassified as to mate-killing.

The second difficulty encountered comes from the fact that occasionally pairs from a mate-killer × sensitive cross fail to manifest mate-killing. Two reasons for the occurrence of such pairs are known and will be presented later. On the other hand, mate-killer × mate-killer and sensitive × sensitive crosses may yield a rare pair showing unilateral death due, of course, to causes other than mate-killing. The large number of unknown cultures to be tested in certain experiments makes it necessary to limit the numbers of pairs of conjugants in each cross as much as feasible. With small groups, occasional discordant pairs assume disturbing proportions. Standards of judgment may be set up in accordance with experience. These will first be set forth and then justified.

The classification of unknown cultures was based upon the viability of six pairs (or nine in a few experiments) isolated from a mixture of the unknown with either a known mate-killer or known sensitive culture. If at least five out of six (or eight of nine) such pairs agreed as to mate-killing or full viability, the unknown was recorded as indicated. If less than these numbers agreed, then the phenotype of the culture was not clearly indicated by the cross and the unknown was scored as " undetermined." Undetermined cultures will be discussed more fully when they are encountered.

The above method of classification has been verified in two ways. First, it is possible to score cultures as mate-killers or sensitives on the basis of the staining reaction to either Giemsa (SIEGEL 1953) or basic fuchsin. Such classification has never failed to parallel the interpretation given the results of the breeding analysis. Second, throughout the analysis some unknown cultures have been crossed to both mate-killers and sensitives. The classification based upon one cross has always been confirmed by the results of the second.

In general, while there is no absolute assurance that every unknown is correctly classified, the method is valid for detecting similarities and differences between the cultures obtained from a variety of crosses.

RESULTS

Sensitives derived from stock 138MK

The discovery that mate-killers always contain 500 to 2000 Feulgen-positive cytoplasmic bodies called " mu " whereas comparable bodies are never found among sensitives (LEVINE 1953; SIEGEL 1953) formed the starting point for the present study.

It is known (SONNEBORN 1946) that the cytoplasmic factor " kappa," responsible for the killer trait, is lost when killer paramecia are cultured at temperatures of 38.5°C. Somewhat lower temperatures have also been found to reduce the kappa concentration of killer cells. Thus in the first attempt to free mate-killers of mu, a typical culture of stock 138MK, mating type XVI, was placed at 33.4°C for one day and then maintained at 27°C with sufficient food added to permit about one fission per week. After about three and one half months, a sample of the culture was stained and examined. Some animals were found to lack mu. Reisolations from this mixed culture yielded some clones which lacked mu and were sensitive as shown by crosses to 138MK.

In a second attempt to obtain sensitive cultures from stock 138MK three isolations were made from a culture of mating type XV of stock 138MK. These were cultivated at 32°C for six days in serial daily isolation in an excess of food, each undergoing a total of 36 successive fissions during that period. They were thereafter provided with enough food for one fission per day and maintained at 27°C. All three cultures were then found to lack mu and were sensitives. Two of the cultures consisted of amicronucleate animals which multiplied for several months and finally died. The probable explanation of the abnormal nuclear condition and death of these two clones appears below, page 556. The third culture was normal.

The viable sensitive cultures obtained in the ways just set forth have been observed for more than two years and have not reverted to mate-killers. Their mode of origin implies that they are either isogenic with stock 138MK or very nearly so; hence, they will be referred to hereafter as 138S.

The F_1 survivors from crosses of 138MK × 138S are always mate-killers and contain mu. Five F_1 clones were backcrossed to the 138S parental culture and the viable BC clones were tested for phenotype. If the derived sensitive cultures, 138S, possess genes unable to support the mate-killer trait, then

sensitive BC clones might be expected. Of 94 BC clones obtained, 82 were mate-killers. Of the other 12 clones, five were selfers, three showed reduced viability, and four remained undetermined. Since no sensitive progeny appeared, it may be tentatively concluded that the 138S cultures have genes capable of supporting the mate-killer trait but are sensitive because the mu bodies and the mate-killer trait cannot be gene-initiated. If so, mate-killing is cytoplasmically inherited, for the cross 138MK × 138S produces progeny which resemble that parent from which they are cytoplasmically descended.

This interpretation depends upon the evidence that matings between mate-killers and sensitives involve reciprocal fertilization. The next experiments bear on this problem.

Evidence for reciprocal fertilization in crosses involving mate-killing

(*A*) *Evidence from F_2 mortality.* A recognized test for stock diversity in *P. aurelia* is the higher frequency of inviable F_2 clones following an interstock cross than following an intrastock cross. The occurrence of this greater inviability in the F_2, when homozygosity is attained, indicates that the incompatibility is nuclear and that reciprocal fertilization had occurred. Therefore if fertilization of at least the MK parent takes place in crosses between mate-killers and sensitives of diverse stocks, the F_2 should include more moribund clones than an F_2 from crosses involving mate-killers and sensitives of the same stock.

The crosses indicated above were made using for the purpose cultures of 138MK, 138S, and 137S. The F_2 was obtained by autogamy; only clones consisting of the progeny from five to six fissions after two days were scored as viable. Since some clones scored as viable may have proved nonviable had they been observed for longer periods than two days, the data give the maximum viability of each cross. All intrastock crosses, including of course 138MK × 138S, gave 90 to 100 percent groups of viable F_2 clones. When stocks 138MK and 137S were crossed, only 60 to 70 percent of the F_2 clones were viable. These data thus favor the interpretation that surviving F_1 clones from crosses involving mate-killing are stock hybrids.

(*B*) *Cytological evidence of pronuclear transfer.* Amicronucleate conjugants are unable to yield exconjugants that form macronuclear anlagen unless they receive a pronucleus from a normal conjugant. An amicronucleate sensitive culture was crossed to a normal mate-killer and the dying sensitive exconjugants, visibly smaller and less active than the mate-killer exconjugants, were fixed, stained, and examined for macronuclear anlagen. Anlagen were found in some sensitive exconjugants, hence these must have received a gamete nucleus from their mates. Since mate-killers can contribute pronuclei to sensitive amicronucleate conjugants, it is likely that crosses between normal mate-killers and sensitives involve reciprocal fertilization.

(*C*) *Evidence from genic segregation.* If it can be shown that a cross between mate-killer and sensitive cultures produces progeny in ratios expected for genic segregation, then the evidence for reciprocal fertilization in crosses

involving mate-killing would be conclusive. Such a cross would be different than those previously described (LEVINE 1953; SIEGEL 1953) for although each pair would show mate-killing and the surviving exconjugants would be descended from the parental mate-killer culture, some of them would change to sensitives. Moreover, the dependence of the mate-killer trait upon both cytoplasmic and nuclear factors would then be demonstrated.

Five pairs were isolated from a cross between cultures of stocks 138MK and 151S and all showed mate-killing. When the F_1 survivors were backcrossed to 151S all 19 of the pairs isolated showed mate-killing and hence the F_1 clones were mate-killers. But tests on the 19 BC surviving clones disclosed that nine of them were mate-killers and 10 had become sensitives.

Of the 10 sensitive BC cultures obtained from these crosses, one was selected for further study because, unlike the others, it was fully viable. (The basis for the reduced viability generally found among sensitives of mate-killer origin is known and will be described later. Thus these apparent exceptions will be shown to conform in principle to the rule that pairs which show mate-killing produce one viable and one nonviable clone.)

TABLE 1

The results of crossing F_1 (138MK × 176S) clones. MK—mate-killer; Sens.—sensitive; Und—undetermined.

F_1 crossed to	Number of F_1 clones crossed	Total F_2 or BC clones tested	Number of F_2 or BC		
			MK	Sens.	Und
F_1	4	70	49	18	0
176S	6	178	66	55	10
138S	6	47	40	1	0

The viable sensitive culture was again mated with stock 138MK and an autogamous F_2 generation was obtained from an F_1 mate-killer. Although many sensitive F_2 clones appeared, again one had to be selected because others showed large amounts of mortality. This F_2 sensitive, culture 176S, has never reverted to the mate-killer phenotype and was used in the crosses to be described next.

More than 50 surviving F_1 clones from crosses between stock 138MK and culture 176S were tested for phenotype. With the exception of a few clones not positively classified on the basis of available data, all were mate-killers. These F_1 clones were crossed to each other and to cultures 176S and 138S. The results of these crosses are presented in table 1.

Crosses between the F_1 mate-killers yielded no regular unilateral death among the F_2 and usually the phenotype of only one member of each pair was determined. However, both members of 23 pairs were tested for phenotype and found to be alike in 22 cases and in one pair they differed. The most likely explanation for the single exceptional pair is that the mates failed to conjugate (i.e., the pair was cytogamous), self-fertilization producing a mate-killer in one and a sensitive in the other clone. Two selfer F_2 clones and one

F_2 clone which showed reduced viability were not crossed and do not appear in the table.

Most pairs from crosses between the F_1 and culture 176S resulted in mate-killing and the phenotypes of the surviving BC clones were determined. However, other BC progeny were not tested; 14 of these were selfers and 33 showed reduced viability.

The crosses between culture 138S and the F_1 clones also yielded mate-killing. The progeny from one pair selfed and the progeny from five pairs showed reduced viability; these six clones were not tested further.

The fact that approximately equal numbers of mate-killer and sensitive progeny result from the cross of $F_1 \times 176S$ whereas, with the exception of one clone, no sensitives segregate from the cross $F_1 \times 138S$ may be interpreted as evidence for the segregation of a single gene difference between the parental cultures of 138 and 176S. The approximate 3 : 1 ratio obtained among the F_2 from the cross $F_1 \times F_1$ supports this hypothesis. These results confirm the earlier assumption that 138MK and 138S cultures are isogenic with respect to the mate-killer trait.

The single exceptional sensitive clone from the mating of $F_1 \times 138S$ can be explained as the result of cytogamy. Assuming this to be so, since this pair showed mate-killing, it would be a confirmation of other experiments which establish the fact that mate-killing does not depend upon nuclear exchange (SIEGEL 1953).

The results of two crosses disagree with those given in the table and are not included there. The exceptional results can be formally explained as due to either macronuclear regeneration (SONNEBORN 1940), or to the failure of these pairs to undergo reciprocal fertilization. The F_1 cultures could not be studied further because they were discarded before the aberrant results were recognized. Two attempts to repeat these results yielded data agreeing with those which appear in table 1.

Cytological studies of BC progeny

Sixteen BC cultures from the $F_1 \times 176S$ backcross were selected for cytological study. These included eight mate-killer and eight sensitive cultures as determined by the breeding test. The animals of all clones previously classified as mate- killers were found to contain mu bodies; mu was not found among those classified as sensitives. Thus the loss of the mate-killer trait was always paralleled by a loss of the mu bodies. Mu is thus necessary for the expression of mate-killing and when removed by gene substitution (or environmental action) a cell can produce only sensitive progeny. Further, mu cannot be gene-initiated for the 138S cultures contain the same genes as 138MK and yet do not revert to the mate-killer phenotype. It is thus concluded that a gene *m* in culture 176S, recessive to its allele *M* in 138MK and 138S, can cause the loss or destruction of mu bodies when homozygous.

A second and completely unexpected fact emerged from the cytological study of these 16 cultures. The nuclei in all eight of the mate-killer cultures

appeared normal; at the proper time after autogamy mate-killers possessed macronuclear anlagen. But the sensitive cultures all contained some animals which were either amicronucleate or had morphologically abnormal micronuclei. Such abnormal micronuclei take only a pale basic stain and are often larger or smaller than normal nuclei. Apparently some sensitives do have normal micronuclei for, as stated earlier, it is possible to select fully viable sensitives derived from mate-killers. Most of the sensitives are unable to form macronuclear anlagen at autogamy and such cells must either die or undergo macronuclear regeneration. The previously noted low viability among sensitives descended from mate-killers is clearly due to their abnormal nuclear condition.

When sensitives are obtained from mate-killers by either environmental effects (page 552) or by gene-substitution they usually suffer micronuclear damage. This suggests that the loss of mu is related to the nuclear damage. If heterozygous cells which lack mu become *m/m* cultures will they incur similar nuclear damage? Sensitive *M/m* F_1 clones (obtained from crosses between sensitives 138S and 176S) were backcrossed to culture 176. None of the BC clones, some of which were *m/m*, contained cells with abnormal micronuclei. Hence nuclear damage appears when heterozygous mate-killers become *m/m* and must be caused by the loss of mu rather than the genotypic change among such clones.

Cytoplasmic exchange during the conjugation of mate-killers to sensitives

If the conjugants of a mate-killer × sensitive cross exchange large amounts of cytoplasm, one of two events may follow. First, both exconjugant clones may be viable and these will be mate-killers. Second, the pair may show mate-killing, the surviving clone being a mate-killer. (Because of these two types of results, test pairs from an unknown by mate-killer or sensitive culture cross which were known to have exchanged cytoplasm were scored separately.) The basis for the difference between these two results is not yet known and the problem requires further study.

Undetermined, reduced viable, and selfer cultures

The conclusion that stock 138 and culture 176S contain alleles *M* and *m* respectively is dependent upon the validity of the assumption that the undetermined F_2 and BC cultures do not represent a third class of segregants differing in principle from those classified as mate-killers and sensitives. This assumption is based upon the following considerations.

First, if the method of classification of unknowns is changed so as to require agreement among all six of the pairs from a cross of an unknown × 138MK, then the ratios of mate-killer to sensitive progeny remain unchanged but more cultures are scored undetermined. If, on the other hand, unknowns are classified on the basis of agreement between only four or more of six pairs very few undetermined cultures are found and the ratios previously obtained again remain the same.

Second, since undetermined cultures have appeared among progeny from crosses between isogenic as well as diverse cultures, it is unlikely that there is a particular genic basis common to them all.

One cause for undetermined cultures is the accidental loss of pairs in handling. Conjugants which fail to separate soon after fertilization and which exchange large amounts of cytoplasm are also "lost" for they are not comparable to the others of a cross and are recorded separately. The effect of omitting these kinds of pairs is to reduce the number of pairs on which the classification of the unknown can be based. If the number of pairs classified then falls below the standard described, the culture must be scored as undetermined by this test.

Some pairs from a cross between mate-killer and sensitives fail to show mate-killing because they fall apart during the early stages of mating (SIEGEL 1953) or exchange cytoplasm. Although such discordant pairs are rare and usually detected, their occurrence may obscure the classification of an unknown and cause it to be scored as an undetermined culture.

Finally, heterozygous F_2 or BC mate-killers clones could be classified as undetermined if some animals underwent autogamy yielding homozygous mate-killers and sensitives. If as much as 50 percent of a culture originally M/m has passed through autogamy at the time of testing (a higher figure than yet found), calculations show that slightly more than half the time it will be scored as undetermined on the basis of the data from six test pairs. Less than one in 100 such heterozygous cultures will be misclassified as sensitives.

Cultures recorded as of " reduced viability " or as " selfers " are probably neither always mate-killers nor always sensitives and because these types have been found among the progeny of crosses between isogenic cultures they can hardly be considered an exceptional class of segregants.

DISCUSSION

The presence of mu is necessary for a paramecium to be a mate-killer. Although mu cannot arise *de novo,* it is supported by the stock 138 genome. Suitable crosses between this stock and stock 151S (or culture 176S) show that certain genomes are unable to support mu. The data from the several crosses suggest that a gene *M* of stock 138 is dominant to an allele *m* of stock 151S. When a stock 138 mate-killer is crossed to one kind of sensitive (138S), no sensitive segregants appear among the succeeding generations; if crossed to another kind of sensitive (151S or 176S), the following generations contain sensitive segregants.

The same interactions between genes and cytoplasmic factors were reported by SONNEBORN (1943) for the inheritance and determination of the killer trait in *P. aurelia,* variety 4. The killer and mate-killer traits have been compared and contrasted by LEVINE (1953) and SIEGEL (1953).

On the basis of a series of experiments reported elsewhere (SIEGEL 1953), it was suggested that the mechanism of mate-killing by stock 138 involved nuclear damage. In support and extension of this hypothesis, it has been

found that mate-killers deprived of their mu particles by either gene substitution or environmental treatment become sensitives and usually suffer nuclear damage. Perhaps a cell with a certain critical concentration of mu is resistant to intracellular nuclear damage but when the mu concentration is lowered, the resistance is lost. In agreement with this, it has been found that sensitive BC segregants first show signs of physiological abnormality (one indication of micronuclear damage) at about the same time that the mu concentration per cell is rapidly decreasing. In contrast to this, when kappa is removed from killers of varieties 2 and 4, the cells apparently never incur nuclear damage. Preliminary experiments indicate that the rapid depletion of mu occurs between the third and sixth fissions following the genotypic change from *M/m* to *m/m*. CHAO (1953) discovered that when killers of variety 4 become sensitives, the marked decrease in kappa concentration lags eight fissions behind the genotypic change.

The loss or destruction of nuclear material is a well known fact. For example, in some species of the nematode genus Rhabditis sperm degenerate in the cytoplasm of the egg (WILSON 1925). Haploid nuclei disintegrate during nuclear reorganization in Paramecium. The present analysis suggests the possibility that localized changes in the concentration of cytoplasmic constituents may cause these phenomena.

Mate-killing and evolution

The cytoplasm has been generally assigned a passive role in evolution due to the relatively few cases of cytoplasmic inheritance known and to the difficulty in ascribing evolutionary significance to cytoplasmic factors. A consideration of the facts involved in mate-killing has led to speculations concerning the possible role of the mu bodies in evolutionary change.

Suppose a mate-killer population in nature is occasionally able to produce viable isogenic sensitives. Such cells would be devoid of mu, hence physiologically different from the parental mate-killer population. If the new sensitive organisms are not killed by conjugation they might occupy a new niche suited to their particular needs. Thus spatially isolated, genic diversity may accumulate between the two populations. The initial step in this microevolutionary change has been a cytoplasmic " mutation."

Since crosses resulting in mate-killing usually involve reciprocal fertilization and genetic material transmitted to the sensitive cannot be perpetuated, contiguous mate-killer and sensitive populations in nature will be partially genetically isolated. But isogenic sensitive cells produced by the mate-killer population may provide a genetic bridge between the hypothetical populations, offsetting an otherwise unilateral flow of genes.

Curiously, another case of unilateral gene flow in microorganisms, that described by ZINDER and LEDERBERG (1952) for the bacterium Salmonella, is due to the presence of another " cytoplasmic factor," a bacteriophage.

Mu may act as an agent of genic selection within a population of mate-killers. If the gene *m* arises in a mate-killer population by mutation or is intro-

duced by conjugation with a sensitive cell, the heterozygous mate-killers will soon produce approximately equal numbers of exautogamous *M/M* and *m/m* cells. Most of the *m/m* cells will become amicronucleate or will have damaged micronuclei making them not only less viable than normal cells but also unable to successfully transmit the recessive allele to new clones. In this way, mu selects against the *m* allele and all other genes linked to it. If mu is of extrinsic origin and is a symbiont, it has a unique way of abetting its own continuity; it exterminates or "sterilizes" cells unable to support it and which might otherwise have competed with its normal host.

JONES (1951) has postulated that cytoplasmic factors may play a role in the final separation of species and wonders "whether or not they (these factors) are induced by nuclear differences." The mate-killer analysis shows how, in this case, the cytoplasm could influence evolution and adds, furthermore, a means by which separation of species could be brought about by cytoplasmic factors that cannot arise *de novo* under the action of any known genome.

SUMMARY

The mate-killer trait depends upon the presence of cytoplasmic bodies called "mu." Cells lacking mu are sensitives and will die following conjugations with mate-killers. Mu cannot arise *de novo* and in certain genomes it is lost or destroyed.

Normal reciprocal fertilization occurs in crosses between mate-killers and sensitives.

When mate-killer cells are transformed to sensitives the change is usually accompanied by micronuclear damage. This suggests an intracellular effect of mu similar to its intercellular effect.

The possible evolutionary significance of mate-killing is discussed and the role of mu in the isolation and diversification of populations within variety 8 of *P. aurelia* is discussed.

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THE DIVERSE MATE-KILLERS OF PARAMECIUM AURELIA, VARIETY 8: THEIR INTERRELATIONS AND GENETIC BASIS[1]

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THIS paper deals in large part with the basis of hereditary differences in a trait which is controlled by the cooperative action of a nuclear gene and visible cytoplasmic factors. As in DIPPELL's (1950) comparable analysis of hereditary differences in the killer trait, the seat of specificity of the differences in the trait here reported upon also appears to lie in the cytoplasm, not in the genes. In general this trait is like the killer trait (SONNEBORN 1943, 1947b; PREER 1948, 1950), but in particular its distinctions are so remarkable that it has been designated by the special term "mate-killer" (SIEGEL 1953a, 1953b).

Siegel discovered mate-killing through the analysis of crosses of stock 138 to other stocks of variety 8. In each conjugant pair, the member derived from the other stock dies, but the member derived from stock 138 lives and produces normal progeny. The former are called sensitives; the latter, mate-killers. Mate-killers are distinguished from sensitives by possessing kappa-like Feulgen-positive particles in the cytoplasm which SIEGEL calls mu. As with kappa in the killer trait, mu seems to depend for its maintenance and reproduction, but not for its origin, on a nuclear gene, which SIEGEL calls *M*. The genetics of mate-killers thus appears parallel to the genetics of killers in variety 4, but the conditions for killing are different. The killing action of mate-killers depends upon prolonged pellicular contact and has not thus far been producible by fluids in which the mate-killers have lived. In variety 4, such fluids are active in killing, due to the liberation of a poison, paramecin, by killers; but contact during mating does not result in killing.

While making a survey of the intervarietal breeding relations among the stocks of varieties 4 and 8 (LEVINE 1953), two other mate-killers, stocks 130 and 131, were found in variety 8. Matings of these stocks to known sensitives of variety 8 resulted in the regular survival of the exconjugant clones descended from stocks 130 and 131 and the death of the exconjugants descended

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from the sensitives. These mate-killers also have cytoplasmic mu particles. The first part of this paper will establish that the three mate-killers are different and the last part will report attempts to establish the hereditary basis of these differences.

MATERIALS AND METHODS

The mate-killers employed in this study were stocks 130, 131, and 138, the only three found in an examination of all (11) known stocks of variety 8. The sensitives used included a " natural " sensitive, stock 130-3 (isolated from the same collection bottle as stock 130), and sensitives derived experimentally from the mate-killers by methods to be described below. Each of the latter, by reason of its mode of origin, is probably isogenic with its corresponding mate-killer.

The general methods used in this work are those described by SONNEBORN (1950). Only the methods for detecting mate-killing and for determining the cytoplasmic origin of survivors of pairs showing mate-killing will be described in detail here. Other methods will be described when necessary. Unless otherwise mentioned, all cultures were grown at 26–27°C.

The following procedure was used to detect mate-killing. The two exconjugants of each pair were followed individually by isolating them into slide depressions containing about 0.2 ml of fresh culture fluid. These were examined two days after the completion of conjugation, when the intrastock control exconjugant clones had usually produced five fissions. At this time, in crosses between mate-killers and sensitives, an obvious difference could be found between the two members of each pair of exconjugant clones: one member, like both members of control pairs, had completed five fissions, the other member had not divided or had undergone only one fission. After further feeding (addition of 0.5 to 0.6 ml of fresh culture fluid) and the lapse of one or two more days, the initial difference was found to be magnified: those exconjugants which had completed five fissions at the first examination had usually produced five to six more fissions and had exhausted their food supply. Those exconjugants which had divided only once or not at all were now dead or dying without having undergone any further divisions.

The method most commonly used to indicate the cytoplasmic origins of the survivors of pairs showing mate-killing was to determine the mating type of the majority of the survivors of a given cross and to assign their cytoplasmic origin to the parent of the same mating type. Three considerations justify this method. (1) Little change of mating type occurs following conjugation in variety 8 (SIEGEL 1953a; personal observations). The frequency of change of mating type varies from 0 to 20% in different experiments and averages about 10%. (2) High correlations between the mating type of the survivors and that of the mate-killer parent has already been demonstrated by SIEGEL (1953a) in crosses involving the mate-killer stock 138. (3) A similar correlation was found in the present study both in crosses involving stock 138 and in crosses involving the other mate-killer stocks 130 and 131. As implied by the preceding

statements, the correlation between the mating type of the survivors and mating type of the mate-killer parent is not always perfect. The relatively few exceptions have been shown by SIEGEL (1953a) to be instances in which the cytoplasmic descendants of the mate-killer parent changed mating type; and this has been confirmed in these studies. The cytoplasmic origin of the exceptions was assumed to be the same as the other survivors of a given cross, since whenever tested the exceptions were mate-killers of the parental type.

In crosses between the different mate-killer stocks, the methods of growth and identification outlined above were used along with added tests for distinguishing the diverse types of mate-killers. These tests will be described below.

Siegel's practice of starving all mate-killer cultures for three days following a short period of rapid multiplication has been accepted in order to minimize such variations in the mate-killer effect as he describes for stock 138. The question of the existence of such variations in stocks 130 and 131 and the effects of the rate of reproduction of the mate-killers prior to mating is dealt with in the next section.

THE EFFECT OF THE RATE OF REPRODUCTION OF THE MATE-KILLERS ON THE MATE-KILLER TRAIT

The mate-killers of stock 138 (SIEGEL 1953a) have different intensities of activity on sensitives. The sensitives die without undergoing a single fission after mating with mate-killers that had previously multiplied slowly; but although doomed to eventual death, they may live and divide for a considerable period after mating with mate-killers that had previously multiplied rapidly. In either case, death is inevitable. As will be shown, the rate of multiplication prior to mating also influences the intensity of the action on sensitives by mate-killers of stocks 130 and 131. The action of slowly grown mate-killers of

TABLE 1

The effect of growth at different rates prior to conjugation on the mate-killing activity of stock 130. Character of resulting exconjugant clones from cross of mate-killers of stock 130 XV, after growth at different fission rates for four days, to stocks 131 sensitives, 130-3 sensitives, and 130 mate-killers.

Stock 130 XV mate-killer	Results of crosses to:								
Fissions per day for 4 days prior to mating	131 sensitives			130-3 sensitives			130 mate-killers		
	V-V	V-VR	V-D	V-V	V-VR	V-D	V-V	V-VR	V-D
0.5			9			9	9		
1.0			9			9	9		
1.5			9			9	9		
2.0		2	7		9		7	2	
3.0		3	5		9		5	4	
4.0		9			9		9		

V-V = Both exconjugants of a pair viable; no retardation of fission rate.
V-VR = One exconjugant of a pair viable; the other viable after a retarded period.
V-D = One exconjugant of a pair viable; the other died.

these stocks is much the same as for stock 138, but the action of rapidly grown mate-killers is very different. The latter produce only temporary effects on sensitives which completely recover and yield normal viable clones.

These relations are illustrated in table 1 which presents the results of crossing mate-killers of stock 130, previously grown for four days at different fission rates, to two different sensitives and to mate-killers of stock 130 previously grown at one fission per day. Control of the different fission rates of stock 130 was achieved in test tube cultures by the methods of PREER (1948). In this way, mate-killers were grown at 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 fissions per day, respectively, in different tubes. Each tube culture of stock 130 was initiated with about 4000 animals from one and the same source culture. In table 1 are given only the results of crosses in which the mate-killers of stock 130, grown previously at different fission rates, were mating type XV. Similar results were obtained in other experiments in which mating type XVI of stock 130 was grown at different fission rates prior to crossing.

Mate-killers of stock 130 that had been grown at fission rates of 0.5 to 1.5 fissions per day showed the mate-killer effect in its most extreme form. Their sensitive mates of stocks 130-3 and 131 died before completing two fissions. The mate-killers survived normally.

When the mate-killers were grown at 2.0 fissions per day or more rapidly, their effects on some sensitives were merely transient. This is shown most sharply by the matings of these mate-killers to sensitives of stock 130-3. On the second day after conjugation, one member of each pair had gone through five fissions while the other, in most cases, had not divided. The five fission clones then went on dividing normally. During the third day after conjugation, the other exconjugant of each pair began to divide, eventually reaching a normal fission rate and giving rise to a viable clone. Mating type tests and backcrosses showed the normally dividing exconjugant clones to be derived from stock 130 while the temporarily retarded ones were sensitives from stock 130-3.

The same kinds of effect appeared, but less regularly, in the matings to stock 131 sensitives, when the mate-killer parent had been grown at 2.0, 3.0 and 4.0 fissions per day prior to mating. Some of the mate-killer cells grown at 2.0 or 3.0 fissions per day killed the stock 131 sensitives, but some merely retarded them temporarily. Only when the mate-killers were grown at 4.0 fissions per day did they always bring about temporary retardation in the growth of the sensitive mates without killing them.

It will be noted that the results set forth above were obtained with mate-killers of mating type XV, stock 131. The results obtained with mating type XVI were irregular. This irregularity and its probable cause will be discussed later after presenting certain other facts pertinent to the problem.

The demonstration that the effects of the mate-killers on sensitives differ in dependence upon the prior growth rate of the mate-killers raises the question of whether these changes in the mate-killers are reversible. SIEGEL (1953a) showed that they are reversible in stock 138 and the observations on stocks 130 and 131 are in agreement with his. Mate-killers of stocks 130 and 131 that

produced only temporary retardation in the growth of sensitives, by reason of the prior rapid growth of the mate-killers, killed sensitives if the mate-killers were later starved for two or three days and were then given enough food for only one fission before they were mated to the sensitives.

The preceding discussion of the mate-killers of stocks 130 and 131 brought out a number of points of resemblance to the mate-killers of stock 138. The enumeration of these similarities should not, however, be allowed to obscure the important difference as to the effects of rapidly grown mate-killers. In stock 138, rapidly multiplying mate-killers doom the sensitives to ultimate death after a period of multiplication, apparently by the reason of irreversible damage to their nuclei (SIEGEL 1953a). In stocks 130 and 131, rapidly reproducing mate-killers produce brief, transient effects on the early reproduction of sensitives and recovery is rapid and complete. Whatever damage is done to sensitives by these two rapidly grown mate-killers must be temporary and reparable; and whether it is on the nuclei is not known.

Since rapidly grown mate-killers of stocks 130 and 131 do not kill sensitives, the question arises as to whether they are sensitive to slowly grown mate-killers which are capable of killing sensitives. As is evident from table 1, the matings of rapidly grown mate-killers to slowly grown ones usually result in the normal survival of both. However, six exceptional pairs are listed in table 1; one exconjugant clone of each of these pairs was temporarily retarded in its growth rate and the other reproduced at the normal fission rate. Does this mean that rapidly grown mate-killers can sometimes respond as sensitives when mated to slowly grown mate-killers? A more complete answer to this question can be given after considering material to be presented in the next section. The discussion presented here is limited to the exceptions in table 1.

Three lines of evidence warrant the conclusions that these were not examples of mate-killer action, but were cases of temporary retardation in fission rate due to other unknown causes. First, the divergence in fission rates between the exconjugant clones in these six pairs was not as great as between the pair members of matings to sensitives. Second, if the retardation was an effect of mating-killing it would be expected to occur even more markedly in the matings to stock 130 mate-killers grown at 4.0 fissions per day; this did not happen. The progeny of the nine pairs in this cross reproduced normally. Third, this retardation was not found in other similar experiments. Thus, it appears that under the conditions of the experiment there was no effect of the more slowly grown mate-killers on the more rapidly grown cultures.

Since the more rapidly mate-killers are grown prior to mating, the weaker the effect they have on sensitives, the question arises as to whether long continued rapid growth could result in failure to affect sensitives at all. The next section examines this possibility.

THE TRANSFORMATION OF MATE-KILLERS INTO SENSITIVES

Growth of mate-killers of stocks 130 and 131 at rapid rates eventually results in the loss of activity on sensitives; moreover, the mate-killers them-

selves become sensitives. What is the physical basis of this transformation? It will be shown that the transformation of mate-killer into sensitive results from the loss of mu particles.

The loss of the mate-killer trait in stocks 130 and 131 has been observed following growth at the maximum fission rates at 20°, 27° and 32°C. Only the experiment carried out at 27°C is reported here; the results obtained at the other temperatures are essentially similar.

The maximum fission rate of slightly more than 5.0 fissions per day was attained in daily isolation cultures (SONNEBORN 1950) started with single mate-killer animals of stocks 130 and 131 grown in slide depressions. Each day, one animal was reisolated to start a new subculture and the remainder of the culture was retained until the food was exhausted (i.e., after about ten fissions) and the animals were starved for three days. Then enough food was added to permit one additional fission and the animals were tested on the following day for mate-killing activity. This procedure was carried out on the seven successive cultures obtained from seven daily isolations, a complete series of seven being obtained both for stock 130 and for stock 131. From the fission rate of these daily isolation series, it is clear that there had been approximately 5, 10, 15, 20, 25, 30 and 35 fissions at maximal rate in the first to seventh cultures of the series, respectively.

All the animals tested from the first and second cultures in the series were mate-killers. The third cultures in the series from both stocks were mixtures of mate-killer and sensitive animals. Practically all the animals tested in the fourth and all tested in the fifth, sixth and seventh cultures were sensitives. These were killed by mate-killers and did not affect sensitives. Cytological examination disclosed that the animals which had lost their mate-killing activity and were transformed into sensitives also lost the mu particles. The transformation of mate-killers into sensitives accompanied by the loss of mu has been confirmed by SIEGEL (1953b) working with stock 138 mate-killers, using somewhat different methods.

Again the question of the reversibility of these changes in the mate-killers must be raised. It would appear that most of these transformations are irreversible. Sensitives derived from stocks 130 and 131 have been under observation for almost two years and still have not recovered any mate-killing activity or mu particles. Neither have the stock 138 sensitives produced by Siegel reverted to mate-killers.

As these sensitives were derived from the mate-killers by environmental means and not by genic substitution, they should be isogenic with the respective mate-killers. Evidence will be presented showing that these sensitives are genically capable of supporting the mate-killer trait, ruling out the alternative that loss of mu was due to genic mutation. The correlation of loss of mu with loss of the mate-killer trait, in the absence of any genic change related to this trait, indicates strongly that the mate-killer trait cannot exist in the absence of mu. Further, the failure of these transformed sensitives to replenish the mu particles, demonstrates the inability of the genes to initiate the production of the particles.

The facts described and the conclusions drawn from the mate-killer trait agree with, in general, those described for the killer trait (SONNEBORN 1943, 1947a; PREER 1948). PREER has shown that some kappas of variety 2 killers are not able to increase as rapidly as the cells when the latter increase at maximal rate over a wide range of temperatures. Consequently the kappa concentration progressively decreases and the killer character undergoes a series of changes. A small reduction in kappa concentration changes the cell from a strong killer to a weaker one. A greater reduction in kappa results in the loss of killing activity, but the cell remains resistant to paramecin. With still further reduction, the cell becomes sensitive to paramecin. These changes are reversible until the last particle of kappa is removed from the cell; then the animal is irreversibly transformed into a sensitive. Since kappa in stock 51, variety 4, can multiply as rapidly as the cells over a wide range of temperatures, growth at maximal fission rates at these temperatures is not effective in producing sensitives. However, at temperatures between 29° and 34°, the cells can multiply more rapidly than kappa and at still higher temperatures kappa is destroyed. In these ways there can be produced a series of changes similar to that described for variety 2 killers (SONNEBORN 1947a and later abstracts).

The progressive changes in mate-killing activity are similarly interpreted as due to the decrease in the concentration of mu because of its slower rate of reproduction than that of the cells. SIEGEL (1953a), using stock 138, showed that the intensity of mate-killing is directly related to the concentration of mu particles in the mate-killers. Preliminary experiments (SIEGEL personal communication) have not demonstrated any action of slowly grown mate-killers on rapidly grown mate-killers before the latter are completely denuded of particles.

In sharp contrast with the large numbers of sensitives obtainable from stocks 130 and 131, preliminary evidence (SIEGEL unpublished) indicates that sensitives are not as readily derived from mate-killers of stock 138. This may mean that just as different kappas are known to multiply at different rates (SONNEBORN 1947a; PREER 1948; DIPPELL 1950), stock 138 mu may increase more rapidly than mu from stocks 130 and 131.

SIEGEL (1953a) reports that some of the stock 138 sensitives obtained by loss of mu through environmental means, have permanently damaged nuclei or are amicronucleate. SIEGEL interprets the damage to the nuclei as self-inflicted, when the mu concentration falls below a critical level. He has shown that the damage to the micronuclei of "doomed" sensitives, produced as a consequence of mating with rapidly grown mate-killers of stock 138, can also be correlated with a decrease in the concentration of mu in the mate-killers. The phenomena in sensitives obtained from mate-killers of stocks 130 and 131 are in marked contrast with those reported by SIEGEL for stock 138. The sensitives of the two former stocks show no evidence of being amicronucleate or of possessing damaged nuclei. This is correlated with similar lack of nuclear damage when sensitives are mated to rapidly grown mate-killers of these two stocks.

The loss of mu as a result of the differential rates of cell and mu reproduction in mating type XV of stocks 130 and 131 requires, as set forth above, at least three days of growth at maximal rate. Indications are that similar results are obtained with even shorter periods of rapid growth of mating type XVI, especially so in stock 131. The period of rapid growth required for the latter may be so short that there is danger of losing mu if these animals are grown at maximal rate for as short a period as one or two days and even if the growth rate is less than maximal. Because of this and because this problem was outside the scope of the present study, it was put aside for later investigation, and type XVI mate-killers of stock 131 were not used in this study except when necessary. However, the more rapid loss of mu from type XVI than from type XV is to be expected in the light of knowledge of kappa in variety 4. CHAO (1953) found that the amount of kappa is twice as great in killers of mating type VII as in killers of type VIII. Since type XVI is the homologue of type VIII, it is not unlikely that it contains much less mu than does type XV. Hence, it might be expected to lose its mu by shorter periods of growth at rapid rates than those required to eliminate mu from mating type XV.

In the discussion up to this point, we have stressed on the one hand the correlation between the presence of mu and possession of the mate-killer trait and, on the other hand, the correlation between the absence of mu and sensitivity to mate-killers. This implies that possession of mu not only determines mate-killing, but also resistance to mate-killing. The implication is correct with reference to the relations between animals of the same stock. However, as will be set forth in the next section, the implication is *not* correct with reference to relations between mate-killers of different stocks.

THE INTERACTION OF THE MATE-KILLERS WITH EACH OTHER

Previous work on varieties 2 and 4 (SONNEBORN 1939, for variety 2; DIPPELL 1950, for variety 4) has shown that different kinds of killers may kill each other. The mate-killers of variety 8 may also, as will appear, affect each other to some extent; but in matings between any two of the three diverse mate-killer stocks, one and only one of the mate-killers is killed, the other survives. Moreover, when the cytoplasmic origins of the survivors are determined, it is found that in each cross one of the mate-killer stocks is regularly resistant to the action of the other, and a "peck-order" of mate-killing emerges. The peck-order, according to strength of mate-killing, is: 138—130—131.

The data to be presented now as evidence for the peck-order are a sample of a larger group leading to the same conclusions. The mate-killers used in these experiments were properly starved to permit the sharpest expression of mate-killing. Reciprocal crosses showed the same results.

Stock 131 is placed at the bottom of the peck-order because it was killed by the other two mate-killers and had no effect on either. Of 30 unilateral survivors of the cross of mate-killers of stock 138 to those of stock 131, 28 were the mating type of the stock 138 parent and two were of the comple-

mentary mating type. Twenty-seven of the survivors of a cross of stock 130 mate-killers to mate-killers of stock 131 were of the mating type of the stock 130 parent and three were not.

Stock 138 is placed higher in the peck-order than stock 130, because in matings between these two stocks the exconjugant clones cytoplasmically descended from stock 138 survived and those from stock 130 died. Forty-four of 50 surviving exconjugants (one from each pair) were of the mating type of stock 138, five were not and the mating type of one was not determined. However, these surviving exconjugant clones, derived cytoplasmically from stock 138, were retarded in their early reproduction in much the same way as were sensitive clones mated to rapidly grown mate-killers of stocks 130 and 131. Two days after the completion of conjugation, these clones had passed through only two to three fissions whereas the intrastock controls had completed five fissions. The retarded clones then recovered the normal fission rate and gave rise to viable cultures.

TABLE 2

Classification of unknown cultures as to grade or type of mate-killing. Alternative characteristics of resulting exconjugant clones from crosses of an unclassified culture to mate-killers of stocks 138 and 130 and to a sensitive.

Testers	Classification of unknowns:			
	Like 138 MK	Like 130 MK	Like 131 MK	Like a sensitive
Stock 138 MK	V-V	VR-D	V-D	V-D
Stock 130 MK	VR-D	V-V	V-D	V-D
Sensitive stock	V-D	V-D	V-D	V-V

MK = mate killers.
V-V = both exconjugants of a pair viable; no retardation of fission rate.
VR-D = One exconjugant of a pair viable after a retarded period; the other died.
V-D = One exconjugant of a pair viable; the other died.

Growing stock 138 and 130 mate-killers under conditions other than those described in this section prior to matings between them might result in the death of both exconjugants. It is conceivable even if highly improbable, that conditions in nature might result in the death of both exconjugants following matings between two adjacent mate-killer populations. Such a lethal interaction, due to the action of mu particles, might act as an isolating mechanism if maintained long enough.

The peck-order provides a means of defining the phenotypes of the mate-killers on the basis of their sensitivity to one another. From the results of crosses to three tester stocks (table 2), any culture can be classified as a sensitive or mate-killer and the grade or type of mate-killing determined. This fact makes a study of the hereditary basis of the different grades of mate-killers possible.

GENIC ANALYSIS OF THE DIFFERENT GRADES OF MATE-KILLING

The analysis of the mate-killer trait has raised three important genetic problems. (1) What is the hereditary basis of the differences between mate-killers and sensitives of the same stock? (2) Are there genic differences between

mate-killers and naturally occurring sensitives in respect to the mate-killer trait? (3) What is the genetic control of the differences in mate-killing as manifested in the peck-order by the three mate-killer stocks?

The first question was answered by the demonstration, for all three mate-killers, that the trait depends on the presence of mu in the cytoplasm. The second question has been partially answered by SIEGEL's (1953b) discovery that animals of sensitive stock 151 not only have no mu, but are genically unable to maintain mu, being homozygous for the recessive *m* gene. The third question is the subject of the rest of this paper.

Genetic control of the grades of mate-killing might be visualized in three ways. (1) Differences in both genes and mu might result in different types. Different genes in the three mate-killer stocks may control different modifications of the mu particles. (2) Mu could be identical in the three mate-killers, different genes determining the different types. (3) The three stocks may be genically identical in the control of mate-killing; the genetic basis of the three diverse mate-killers would then rest in the different mu particles. Should one of the first two possibilities be correct, the mate-killer types would be expected to segregate in Mendelian fashion in appropriate crosses.

The experimental procedure

Three series of three crosses were undertaken to test for the genic determination of the mate-killer diversities. In each series, mate-killers of one stock were crossed to sensitives derived from each of the mate-killers. The sensitive conjugant of each pair died or showed one of the other effects of mate-killers on sensitives, but not before providing the mate-killer conjugant with a set of genes. The action of the hybrid genome on the mate-killer trait was tested by determining the grade of mate-killing of the F_1 according to the scheme outlined in table 2. Finally the same testing procedure was used to check for segregation of the mate-killer types among the backcross progeny of the F_1. The backcross progeny were obtained by crossing the F_1 to the sensitive tester stocks which were used in the original cross. Approximately 40 backcross clones from each of the nine crosses in the three series were individually tested for their grade of mate-killing. The general scheme of the experimental analysis is presented in table 3.

Sensitives, isogenic with the mate-killers, were used so that the genes of the mate-killer stocks could be exchanged without complicating the experiment with the possible mixture of mu particles. Sensitives were derived from the mate-killers of stocks 130 and 131 by the growth rate methods previously described. The author is indebted to DR. R. W. SIEGEL for supplying him with a culture of isogenic sensitives derived from mate-killers of stock 138.

The F_1 mate-killers

The tests of the grades of mate-killing of the F_1 clones can be summarized as follows: The three different genotypes in any one series show the same grade of mate-killing. On the other hand, clones in different series, but hav-

TABLE 3

Breeding scheme for the genic analysis of the control of the grades of mate-killing.

Table 3a. The F_1 crosses.

Series	Mate-killer parents	Sensitive parents		
		A 138	B 130	C 131
I	138 XV	6 prs.	9 prs.	9 prs.
II	130 XV	9 prs.	9 prs.	9 prs.
III	131 XV	9 prs.	9 prs.	9 prs.

*Table 3b. Test for the grade of mate-killing manifested by the F_1 survivors from series I.**

No. of F_1 tested	Crossed to the following tester stocks:		
	138 MK	130 MK	
5 from I-A	9 prs./F_1	9 prs./F_1	138 sens. / 9 prs./F_1
5 from I-B	9 prs./F_1	6 prs./F_1	130 sens. / 9 prs./F_1
3 from I-C	9 prs./F_1	9 prs./F_1	131 sens. / 15 prs./F_1

*Table 3c. Test for the grade of mate-killing manifested by the backcross of clones from series I.**

The backcross clones from	Crossed to the following tester stocks:		
	138 MK	130 MK	
I-A	6 prs./clone	6 prs./clone	138 sens. / 6 prs./clone
I-B	6 prs./clone	6 prs./clone	130 sens. / 6 prs./clone
I-C	6 prs./clone	6 prs./clone	131 sens. / 6 prs./clone

MK = mate-killers.
Sens. = sensitives.
*The F_1 and backcross generation survivors from series II and III were tested for their grade of mate-killing in the same manner as were those of series I.

ing the same genotypes, show different grades; the grade of mate-killing always being the same as that of the parent mate-killer.

The F_1 results are not readily compatible with the hypothesis of genic determination. To explain the fact that hybrids of the same genotype exhibit different types of mate-killing, one would have to make the unlikely assumption (in the absence of a phenomic lag) that the genes involved in the mate-

killer trait are both dominant and recessive to each other under the same conditions. If the mate-killers do indeed have different genes in control of the trait, then the backcross generation derived from the mate-killers should show a segregation of the types. If the genes in control of the mate-killers are the same in the different stocks, then the backcross clones should show

TABLE 4

The grades or types of mate-killing manifested by the backcross clones derived from crosses between the various mate-killer stocks (table 3).

Parental mate-killers	Classification of backcross clones	Sensitive parents used in F_1 and backcrosses		
		A 138	B 130	C 131
Series I 138	No. F_1	5	5	3
	No. Backcross clones	42	42	45
	Phenotype of backcross clones:			
	Mate-killer, parental type	37	15	31
	Mate-killer, other types	0	0	0
	Sensitives	0	0	0
	Undetermined	3	21	9
	Not tested {Died	1	4	1
	Not tested {Selfers	1	2	4
Series II 130	No. F_1	3	5	3
	No. Backcross clones	43	45	45
	Phenotype of backcross clones:			
	Mate-killer, parental type	19	27	20
	Mate-killer, other types	0	0	0
	Sensitives	0	0	0
	Undetermined	7	14	10
	Not tested {Died	15	1	15
	Not tested {Selfers	2	3	0
Series III 131	No. F_1	3	5	3
	No. Backcross clones	42	42	45
	Phenotype of backcross clones:			
	Mate-killer, parental type	31	24	36
	Mate-killer, other types	0	0	0
	Sensitives	1	1	0
	Undetermined	7	8	4
	Not tested {Died	1	6	1
	Not tested {Selfers	2	3	4

the same type of mate-killing as the parental and F_1 cultures from which they are cytoplasmically derived.

The backcross generation mate-killers

Table 4 gives the grades of mate-killing shown by the backcross clones as determined from the crosses to the tester stocks. All clones which died before they could be tested or which were not tested because of selfing (mating between the members of a clone) are listed separately in the table.

Verification can now be given for the statements made earlier that sensitives and mate-killers of the same stocks are genically identical with respect to mate-killing, thus justifying the use of these sensitives as donors of genes. There was no case of segregation of the grades of mate-killing or of sensitives among the backcross progeny of intrastock crosses of mate-killers and sensitives (crosses I-A, II-B, and III-C, table 4). (Ignore for the moment those clones classified as undetermined.) Cross I-A (table 4) confirms SIEGEL's work with stock 138. These crosses served as controls for the crosses among the three stocks.

Two sensitive clones appeared among the backcross progeny of the mate-killers of stock 131 to the other two stocks (series III-A and III-B, table 4), but their occurrence is interpreted as not due to genic recombinations. One of these sensitive clones was mating type XVI. The irregularity of mate-killing among mating type XVI cultures of stock 131 was mentioned above. Since no other sensitive clones appeared in the other series, genic recombination is considered highly unlikely.

The striking thing about the information in table 4 is the remarkable agreement between the mate-killing of the backcross clones and those of the parental and F_1 cultures (again ignoring those clones classified as undetermined). Everyone of these backcross clones was a mate-killer (except for the two sensitive clones from the progeny of mate-killers of stock 131 discussed above) typical of the parental and F_1 mate-killer used. In other words, no segregation of diverse mate-killers occurred.

Before it can be concluded that the absence of segregation is a real phenomenon, it must be demonstrated that the backcross clones classified as undetermined do not represent a class of segregants and that the widespread occurrence of cytogamy (pairing of opposite mating types as in conjugation without the exchange of nuclei taking place between the mates) is not masking the presence of genic differences. These considerations will now be discussed and evidence presented minimizing their importance.

The undetermined backcross clones as a factor in the lack of segregation

A backcross clone was classified as a particular mate-killer if no more than one of the six pairs in any of the three matings to testers gave aberrant results. If two or more pairs gave aberrant results, it was decided to waive judgment and classify these clones as undetermined types. By aberrant is meant results in which not all the pairs in a mating show the same pattern of viability. For instance, a backcross clone cytoplasmically descended from mate-killers of stock 130 reacted as a stock 130 mate-killer in the matings to stock 138 and 130 mate-killer testers. However, in the mating to the sensitive testers only four pairs showed unilateral survival, both exconjugants of the other two pairs were nonviable. Another type of aberrant result was the normal survival of both members of a pair in two or more pairs of a test of six, when the other pairs exhibited the typical mate-killer reaction. Both of these backcross clones were classified as undetermined.

Three lines of evidence suggest that the undetermined backcross clones of the first type are not segregants demonstrating genic control of the mate-killer types.

(1) A regularity in the proportions of the undetermined clones in the different crosses would be expected if they represent segregants. No such regularity was evident. Neither was there any regularity in the occurrence of lethality in the matings to the testers which resulted in most of the undetermined clones being classified as such.

(2) An examination of table 4 shows that the majority of the undetermined clones were found in the crosses involving stock 130. Most of the lethality which resulted in the undetermined clones was probably traceable to the sporadic mortality occurring in intrastock 130 crosses rather than to segregation of new types of mate-killers. A large number of intrastock 130 matings were followed in the course of the experiment as controls for the matings to the testers and a great deal of nonviability occurred. Twenty-two sets of six pairs each were followed from the matings between the two mating types of stock 130, both being mate-killers. Five of these would have to be classified as undetermined because of nonviability among the exconjugant clones. All six pairs of one set were aberrant. Six sets of six pairs each from the mating of the two mating types of stock 130 when both were sensitives were similarly followed. Two of these six sets would be classified as undetermined because of nonviability obviously not connected with mate-killing since both conjugants were sensitives. In the matings between stock 130 mate-killers and sensitives, 13 sets of six pairs each were observed. Two of these would be undetermined as some of the mate-killer conjugants were nonviable in addition to the sensitives. The contention that the sporadic mortality in stock 130 is responsible for most of the undetermined clones is supported by the fact that almost a third of the backcross progeny of the intrastock 130 cross (cross II-B, table 4) were undetermined because of excessive mortality in the crosses to testers. Segregation of new types of mate-killers would hardly be expected in this intrastock cross.

(3) The cross of stock 138 mate-killers to stock 130 sensitives (cross I-B, table 3) was repeated and 10 backcross clones were tested for their grade of mate-killing. Eight were stock 138 mate-killers and two were undetermined by our standards. The frequency of undetermined clones in this experiment is significantly different from that of the undetermined clones in cross I-B (table 4): 2 out of 10 as compared to 21 out of 42. This indication that the frequency of the undetermined clones may be variable, would not be expected of a genically controlled trait. Use of cultures which do not show intrastock mortality should greatly diminish the frequency of these undetermined clones.

Very few clones were classified as undetermined because of viability of both members of a pair where it was not expected. The few cases which did occur are explicable on any of the following three possibilities: (1) mating between the members of a clone, (2) both conjugants of a pair becoming mate-killers as a result of cytoplasmic exchange (SIEGEL 1953b), or (3) by the

separation of the mates before the completion of conjugation and the transmission of the lethal effect (SIEGEL 1953a).

Cytogamy as a factor in the lack of segregation

Should cytogamy be of widespread occurrence in crosses to mate-killers, the lack of segregation would be due to the failure of reciprocal fertilization instead of the presence of the same genes for mate-killing in the three stocks. Three lines of evidence minimize the importance of cytogamy in this experiment. First, SIEGEL (1953b) demonstrated reciprocal fertilization in crosses of stock 138 mate-killers to stock 151 sensitives by Mendelian segregation of mate-killers and sensitives in the backcross progeny. Second, a considerable proportion of nonviability is always characteristic of the F_2 by autogamy after interstock crosses in *P. aurelia* and is itself an indication of true crosses in F_1. This was always obtained following crosses among the three stocks. Third, consistent results were obtained for all F_1 clones followed in each cross. Thus cytogamy cannot be an important factor in these experiments.

DISCUSSION

The genetic control of the different grades of mate-killing

The results of the crosses presented in the previous section lead to the conclusion that there are no simple gene differences between the three mate-killer stocks in the maintenance of the mate-killer trait and in the control of the specificity of mate-killing. (This conclusion assumes that a very long phenomic lag is not operating.) It should be pointed out that the relatively small number of backcross clones tested does not rigorously exclude the control of mate-killing specificity by multiple genes, any one of which could be effective in perpetuating the parental type of mate-killing. This could be excluded by demonstrating the direct control of the grade of mate-killing by the mu particles.

DIPPELL (1950) analyzed five mutant killers from two stocks of variety 4 and established that the genes in these mutants are the same in respect to the killer trait. She further demonstrated that the differences between the mutants are due to differences between the kappa particles. This was done by the dilution of the kappa concentration to a single particle per cell, followed by slow growth of the animals to permit cells to regain the original kappa concentration. It was found that some mutant killers contained two different types of kappa particles, each with a particular phenotype associated with it. In all she found three distinct forms of kappa particles, which she concludes must have arisen by mutation of one kind of particle to another kind.

It is not known whether the populations of mu in the three mate-killers are homogeneous or mixed. The determination of the homogeneity of the populations of mu may be very difficult due to the aggregation of the particles (SIEGEL 1953a for stock 138 and personal observations on stocks 130 and 131).

The dilution to a single particle per cell depends on the random distribution and dilution of the particles. If the distribution of the mu particles is markedly nonrandom, it may prove difficult or impossible to dilute the concentration to one particle per cell.

Should the mate-killers not lend themselves to the type of analysis carried out by Dippell on the killers, evidence as to the role of particles in determining the specificity might come from mixing mu particles during crosses between the mate-killers (by inducing cytoplasmic exchange between the mates). It might be expected that the F_1 clones produced would be different from those described in the genic analysis experiment.

The evidence that the genes play no role in the control of the grades of mate-killing, DIPPELL's findings for the killer trait, and the discovery that the mu particles of the different mate-killers are morphologically distinguishable (SIEGEL and PREER personal communication) all speak strongly for the mu particles controlling the specificity of the mate-killer phenotypes.

Stock differences in sensitivity to mate-killing

The peck-order of mate-killing demonstrates that not all the mate-killers are equally sensitive to other mate-killers. There is some evidence that not all the sensitive stocks are equally sensitive to a particular mate-killer. Evidence for this contention is given in table 1. Stock 130-3 sensitives were only temporarily retarded in fission rate by the same stock 130 mate-killers which killed stock 131 sensitives. Results of this sort were frequently seen in these same crosses. Death of stock 130-3 sensitives and survival of stock 131 was never observed. This difference in resistance to mate-killing may be due to genetic differences between the two stocks or to the phenomenon discovered by AUSTIN in connection with differences in sensitivity to killing. AUSTIN (1951) found that different serotypes of the same stock may vary in sensitivity to paramecin killing. The observed difference in sensitivity to mate-killing may be a manifestation of such a serotype effect. A careful study of variety 8 sensitives should be made in this respect.

Mate-killers and killers

The similarities and dissimilarities of the mate-killer and killer traits have been mentioned throughout the paper and need not be repeated. Only a few points previously not mentioned will be discussed.

The particles determining one trait offer no protection against the action of the other. The variety 8 mate-killers are killed in typical fashion by the variety 4 killers. In intervarietal matings between varieties 4 and 8, both exconjugants of a pair undergo four to six fissions before dying if the variety 8 stock is not a mate-killer. In intervarietal matings between mate-killers and killers or sensitives of variety 4, one member of a pair undergoes the expected four to six fissions whereas the other dies without dividing more than once. The exconjugant which dies sooner is the variety 4 killer or sensitive which has been stricken by the mate-killer.

Attempts to determine whether maintenance of the mate-killer and killer traits is dependent on the same locus have so far proved inconclusive. Attempts to introduce kappa particles into variety 8 during conjugation have not been fruitful because of the high mortality occurring in the intervarietal crosses (LEVINE unpublished). LEVINE and SIEGEL (unpublished), using SONNEBORN'S (1950) infection technique, have tried unsuccessfully to infect variety 8 with kappa by exposing stock 138 sensitives of the M genotype to breis of killer animals. Using the same technique, variety 4 sensitives with the dominant gene, K, were exposed to breis of mate-killers of stock 138, again with no success. Efforts to combine the particles of killers and mate-killers should be continued, since if accomplished, not only could the question of genic control of the maintenance of the traits be settled, but studies on competition and genetic exchanges between the particles as are found in the bacteriophages (LURIA 1947) might become feasible.

SUMMARY

Stocks 130, 131, and 138 of variety 8, *Paramecium aurelia,* have been examined for hereditary differences in the mate-killer trait. This trait is known to be controlled by the cooperative action of a nuclear gene and visible cytoplasmic particles called mu. Sensitives mated to slowly reproducing animals of these stocks regularly die before completing two fissions. The exconjugants cytoplasmically descended from stocks 130, 131 and 138 produce viable cultures. When mated to rapidly grown mate-killers of stock 138, sensitives produce a limited number of daughter cells, all of which are doomed to die. In contrast, sensitives mated to rapidly grown mate-killers of stocks 130 and 131 are only temporarily retarded in their early reproduction, then recover and produce normal viable cultures. Prolonged rapid growth of mate-killers of stocks 130 and 131 results in the loss of mu and in the irreversible transformation of these animals into sensitives.

When crossed to one another, the mate-killers exhibit a system of striking interactions. Each cross exhibits the mate-killing reaction: the exconjugants from one of the stocks regularly die and the others survive. This system of interactions gives rise to a "peck-order" of mate-killing which according to strength of mate-killing is: 138—130—131.

A breeding analysis has failed to demonstrate genic control of the grades of mate-killing as manifested in the peck-order. This strongly suggests that the seat of specificity of the differences in mate-killing between the three stocks resides in the cytoplasmic particles.

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INDUCTION OF INSTABILITY AT SELECTED LOCI IN MAIZE

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IN previous reports (McCLINTOCK 1950, 1951), studies of the origin and expression of genic instability at a number of known loci in the maize chromosomes were summarized. It was concluded that changes in genic expression result from chromosome alterations at the locus of a gene and these are initiated by units other than those composing the gene itself. The mutations are considered, therefore, not as changes in the potentials of genic action, but rather chromosomal modifications at the locus effecting the kind and the degree of genic expression. The extragenic chromatin units have specificity in that differences among them may be recognized. Each exerts a specific type of control of the action of the gene with which it becomes associated. These units may be transposed from one location to another within the chromosome complement. When incorporated at a new location, each expresses its mode of control of the action of the associated gene, and in a manner similar to that which occurred at the former location. These conclusions have been supported by extensive examination of the action of one particular system that has modified genic action at a number of different loci. It is the so-called Dissociation-Activator (*Ds-Ac*) two-unit system.

Both *Ds* and *Ac* are single chromosomal units. Their locations in particular chromosomes may be determined by linkage relations to other established markers. Each, however, may move from one location to another within the chromosomal complement. Such transpositions of *Ds* or *Ac*, or both, may occur in a few sporogenous cells. Consequently, a few gametes may be formed with *Ds* or *Ac*, or both, located at new positions. Following such transposition, each remains at the new location until, in a subsequent cell or plant generation, transposition to another location again occurs. Either may be inserted at various locations within the complement. It has been determined that the process of transposition involves some initial change at either *Ds* or *Ac* that can result in chromosome breaks and fusions. It has also been learned that in order for transposition of *Ds* to occur, *Ac* must be present in the nucleus. Besides transposition, there are other known consequences of events initiated by *Ds* and again, these appear only when *Ac* is also present in the nucleus. They are dicentric chromatid formation, deficiency, duplication of segments, inversions, ring-chromosomes and reciprocal translocations between chromosomes. In all of these cases, the chromosome breaks are produced as the consequence of some initial change involving the *Ds* unit.

When *Ds* is transposed to the locus of a known gene, it may immediately or subsequently affect its action. This is expressed either by partial or complete inhibition, or by a previously unrecognized type of altered genic expression. As long as *Ds* remains in this position, genic action is subject to further change. It has been possible to determine that the subsequent changes are reflections of alterations occurring at the locus, and these are initiated by *Ds*. No *Ds*-initiated changes will occur, however, unless *Ac* is also present in the nucleus. If *Ac* is absent, no further modification of genic action occurs. The mutation present at the time of removal of *Ac* will be stable in expression in subsequent generations until *Ac* is again introduced into a zygote. In some cells of the plant arising from this zygote, alterations at the locus of the gene, initiated by *Ds*, again will occur. Some of these may result in further modifications of genic action—that is, new mutations. Both the initial mutation and subsequent mutations are expressions, therefore, of interaction between *Ds* and *Ac*. Thus, these two chromosomal units comprise a nuclear system capable of controlling genic expression; *Ds* initiates changes in genic expression and *Ac* controls when they will occur. Since *Ds* may enter various locations in the chromosome complement, *Ac*-controlled mutability can be expected to arise at a number of different loci of known genic action. In previous reports, the origin and subsequent behavior of *Ac*-controlled mutability at the *C*, *Bz* and *Wx* loci in the short arm of chromosome 9 were described. It is the purpose of this paper to indicate that, in conformity with expectation, *Ac*-controlled mutability can appear at other loci of known genic action. It will also be shown that this type of control of instability may be obtained at selected loci.

To test the prediction that genic instability under the control of *Ac* may be obtained at selected loci, two, considered to be particularly favorable for initial tests, were selected. These are the locus of A_1 in chromosome 3 long arm, and that of A_2 in chromosome 5 short arm. The genetically active components at these two loci affect anthocyanin pigment formation in both the plant tissues and in the aleurone layer of the kernel. The known recessive alleles are designated a_1 and a_2. When either is homozygous, no anthocyanin pigment is developed in the plant tissues or in the aleurone layer. Both recessives are stable in the presence of *Ac*; tests designed to show whether or not mutations of a_1 or a_2 would occur when *Ac* was present in the nucleus were negative. Consequently, it was possible to perform the following experiment. A number of plants were grown that had a *Ds* unit located in the long arm of one chromosome 5 and an *Ac* unit located in another chromosome of the complement. All plants were homozygous for A_1 and Sh_2 and for A_2 and Bm_1. (Sh_2, normal development of endosperm; sh_2, shrunken endosperm, located a quarter of a crossover unit distal to A_1. Bm_1, colorless secondary cell walls in plant tissues; bm_1, brown mid-rib, brown color in secondary cell walls; located 6 crossover units proximal to A_2 and in same arm of chromosome 5.) The silks of some of these plants received pollen from plants homozygous for a_1, sh_2 and A_2. The silks of other plants received pollen from plants homozygous for a_2, bm_1 and A_1. None of the plants used as pollen parents carried *Ac*. The ears resulting from

these crosses were examined to determine if any kernels exhibiting variegation for aleurone color were present.

If a sufficiently large number of ears were obtained from the described crosses, one or more variegated kernels should appear. This conclusion is based on the following reasoning. In plants carrying *Ds* and *Ac*, transposition of *Ds* may occur in a few sporogenous cells. Should one such transposition insert *Ds* at the locus of A_1, total or partial inhibition of A_1 action could result. The gametophyte and consequently the gametes derived from this sporogenous cell could carry an A_1 locus with a *Ds*-initiated altered capacity for action. Should *Ac* also be present, further *Ds*-initiated changes at the locus could occur in some cells during development of the kernel that arises from functioning of this gametophyte. In the progeny of such cells, these subsequent changes in A_1 action could be expressed if the pollen parent had contributed the recessive, a_1, which is stable in the presence of *Ac*. The aleurone layer of the mature kernel would exhibit a variegated pattern of anthocyanin pigmentation, either with respect to presence and absence of pigmentation, or to intensities of pigmentation, or both. This kernel could then be removed from the ear, a plant grown from it and tests conducted with this plant and its progeny to determine whether or not the variegation is an expression of instability at the A_1 locus and, if so, whether this instability is *Ac*-controlled. In a similar manner, alterations at the A_2 locus that arise in the *Ds* and *Ac* carrying plants could be detected if the pollen parent contributed the stable recessive, a_2. In order to establish that the unstable state in either case originates in the *Ds* and *Ac* carrying plant, it is necessary for one of the parents to introduce a second, closely linked genetic marker. In progeny tests, linkage of unstable A_1 with Sh_2 or of unstable A_2 with Bm_1 would indicate inception of instability in the *Ds* and *Ac* carrying parent plant.

Seventy-one ears were obtained from the cross in which the pollen parent had contributed a_1. One kernel was found that clearly exhibited variegation for aleurone color. There were no colorless kernels on any of these ears. Among the 120 ears obtained when a_2 had been introduced by the pollen parent, three kernels exhibiting variegation for aleurone color appeared, each on a different ear. Again, there were no colorless kernels on any of these ears. Plants were grown from all four variegated kernels and tests initiated with each to determine the nature of the instability being expressed. The progeny, in turn, were further tested. From these studies, the following was determined. In the variegated kernel that had received a_1 from the pollen parent, and in the plant derived from it, instability of genic action at the locus of A_1 was being expressed. The alteration responsible for this instability occurred at the locus of A_1 in one of the chromosomes 3 of the *Ds-Ac* carrying female parent. Mutations at this modified A_1 locus, designated $a_1^{m\text{-}4}$, occur only when *Ac* is present. In the plants derived from the three variegated kernels that appeared when a_2 had been the pollen parent, the nature of the alteration at A_2 could be determined in only two of them. This was because in one of them, the chromosome 5 contributed by the *Ds-Ac* carrying female parent was not transmitted to the next

generation either through pollen or egg. Thus, no definite conclusions may be drawn concerning the nature of the instability, presumably at the A_2 locus, that was exhibited in the kernel from which this plant arose. In the two remaining plants, it could be shown that it was the A_2 locus in the chromosome 5 contributed by the female parent that had been modified. In one plant, the mutations occurring at this locus proved to be *Ac*-controlled. This mutable locus is designated $a_2^{m\text{-}4}$. In the second plant, the mutations were not *Ac*-controlled. This mutable locus is designated $a_2^{m\text{-}3}$.

Some of the methods used to determine whether or not mutation at a particular known locus is *Ac*-controlled will be outlined. For illustrative purposes, the A_1 locus has been chosen. Four independent inceptions of instability at A_1 have been detected in the Cold Spring Harbor cultures. Two of them, $a_1^{m\text{-}1}$ and $a_1^{m\text{-}2}$, are not *Ac*-controlled. Two of them, however, $a_1^{m\text{-}3}$ and $a_1^{m\text{-}4}$, are *Ac*-controlled. Both of the latter had their inception in plants homozygous for the normal A_1 locus and in which both *Ds* and *Ac* were present. The designation $a_1^{m\text{-}4}$ was given to the case whose origin was outlined above. In this report, the methods used to analyze the factors responsible for mutation at $a_1^{m\text{-}4}$ will be considered. These same methods, however, have been used to analyze all cases of *Ac*-controlled mutability. It is because of the very close linkage of Sh_2 to $a_1^{m\text{-}4}$, allowing the latter to be readily followed in progeny tests, that instability at the A_1 locus has been selected for illustrative purposes.

In considering *Ac*-controlled mutability, the following facts should be kept in mind. Mutations will occur only when *Ac* is present in the nucleus. In the absence of *Ac*, the modified genic action at the locus is stable. Instability, however, will return if *Ac* is again introduced into a nucleus having this modified locus. *Ac* controls when mutations will occur; the higher the dose of *Ac*, the later the time during the development of a tissue when mutations at the affected locus will occur. *Ac* is inherited as a single unit. Transpositions or alterations of *Ac*, however, may occur in a few sporogenous cells resulting in loss to one of two sister cells, change in location of *Ac* in the chromosome complement, or change in dose action (change in state). Some of the gametes produced by plants carrying *Ac* will be derived from cells in which such events occurred. Evidence for these statements will appear in the descriptions of the tests conducted with $a_1^{m\text{-}4}$.

The pattern of variegation in the original kernel having $a_1^{m\text{-}4}$ resembled that expected of mutable a_1. Within the aleurone layer, many small areas of color appeared in a colorless background. A plant was grown from this kernel in the greenhouse during the winter of 1951–52, and given the culture number 6110. This plant was: (1) self-pollinated, (2) crossed to two plants having no *Ac* factor that were $a_1\ Sh_2/a_1\ sh_2$, (3) crossed to a plant that was $A_1\ Sh_2/a_1\ sh_2$ and carrying a single *Ac* factor, and (4) crossed to a plant with a genetic constitution designed to test for the presence of *Ac* in plant 6110. The nature of the *Ac* tester stocks will be described shortly. The number of crosses conducted in the greenhouse was limited. The results of these crosses did indicate, however, that plant 6110 carried a newly arisen alteration at the A_1 locus; and

that this alteration was responsible for the expressed instability. The constitution of plant 6110 was a_1^{m-4} Sh_2/a_1 sh_2 and it carried one *Ac* factor. The evidence also suggested that the mutations occurring at a_1^{m-4} were *Ac*-controlled. From these greenhouse crosses, kernels with appropriate constitutions were selected and plants obtained from them in the summer of 1952 in order that more adequate tests of the nature of the instability could be conducted.

The self-pollinated ear of plant 6110 produced 228 kernels with the following appearances: 7 completely colored, Sh_2: 129 variegated (exhibiting areas of color in a colorless background), Sh_2: 49 colorless, Sh_2: 43 colorless, sh_2. In the crosses to plants that were a_1 Sh_2/a_1 sh_2 and in which no *Ac* factor was present, 888 kernels were produced. They could be segregated into the following classes: 13 completely colored, Sh_2: 225 variegated, Sh_2: 438 colorless, Sh_2: 212 colorless, sh_2. In the cross to the plant that was A_1 Sh_2/a_1 sh_2 and had 1 *Ac* factor, 273 kernels were produced: 145 completely colored, Sh_2: 1 completely colored, sh_2: 29 variegated, Sh_2: 17 colorless, Sh_2: 81 colorless, sh_2. The several distinctive patterns of colored spots exhibited by the variegated kernels derived from this latter cross suggested *Ac*-control of mutability at a_1^{m-4}. These resembled the patterns produced with single to triple doses of *Ac*. Kernels with these different doses of *Ac* were expected to be present on this ear because each parent carried one *Ac* factor. Other evidence also suggested that the mutations were *Ac*-controlled. This appears in the ratio of variegated to nonvariegated kernels resulting from the cross of plant 6110 to the plants that were a_1 Sh_2/a_1 sh_2 and had no *Ac*. If *Ac* were not linked to a_1^{m-4}, only one-half of the a_1^{m-4} Sh_2 carrying gametes produced by plant 6110 would also carry *Ac*. And, if *Ac* controls mutation at a_1^{m-4}, then in only one-half of the a_1^{m-4} Sh_2 carrying kernels could mutations occur. Since a_1^{m-4} and Sh_2 are very closely linked, the expected ratio of kernel types from the described cross should be: 1 variegated, Sh_2: 2 colorless, Sh_2: 1 colorless, sh_2. The observed ratios were: 225 : 438 : 212, which is a close approximation to this expectancy.

Before presenting the evidence that establishes *Ac*-control of mutation at a_1^{m-4}, the phenotypes produced by mutation will be described. The initial change at A_1 resulted in complete inhibition of anthocyanin pigment formation in the aleurone layer of the kernel and in the plant tissue. Subsequent mutations occur. Two main classes which result in pigment formation can be recognized. In one class, the mutations reestablish the original phenotypic expression of A_1: deep pigmentation in aleurone and plant. In the aleurone layer, the borders of such mutant areas are not precisely defined because of diffusion of pigment forming substances into the surrounding nonmutant cells. This produces " diffusion rims " about these mutant areas. Within the second class, the mutations give rise to a graded series, with respect to intensity of anthocyanin pigmentation; from light to relatively dark shades. The borders of areas having these mutations are sharp. Diffusion rims, characteristic of the first class of mutation either are not present or are very weakly expressed. The phenotypes exhibited by the kernels carrying germinal mutations also express these same

two main classes of mutation. In these cases, however, the color intensity in any one kernel is the same throughout the aleurone layer. The plant tissues likewise show a graded series of intensity of pigmentation among the mutant areas. Marked changes in state of a_1^{m-4} that result in altered relative frequencies of the various types of mutation, were rarely observed. This is in contrast to a_1^{m-1} and a_1^{m-3}, both of which have produced many modified states, each exhibiting a particular type or types of mutation and/or frequency of type.

In order to determine that *Ac* controls mutation at a_1^{m-4}, several types of tests must be applied and positive results obtained from each. These are: (1) establishment of the presence of *Ac* in plants showing mutations at a_1^{m-4}; (2) establishing that a_1^{m-4} is nonmutable in the absence of *Ac* but that return to mutability will occur when *Ac* is again introduced into the nucleus; (3) establishing in this case that it is the same *Ac* factor known to produce breaks at *Ds* and to control mutations at other loci of known genic action, which is responsible for controlling mutation.

The presence of *Ac* can be detected because it produces breaks at *Ds*, wherever it may be located, or mutations at some loci of known genic action (see table 6). In order to be able to test readily for the presence of *Ac* in a particular plant, it has been necessary to develop so-called *Ac* tester stocks. Many of them utilize genetic markers in the short arm of chromosome 9 affecting the phenotype of the endosperm of the kernel, and also *Ds* at its standard location in this arm. For illustrative purposes, the test procedure with one such stock may be described. This stock is homozygous for the chromosome 9 endosperm markers *I*, Sh_1, *Bz* and *Wx*, and for *Ds* at its standard location, proximal to *Wx*. (*I*, inhibitor of aleurone pigment formation; dominant to allele *C*, required for aleurone color development. Sh_1, normal development of endosperm; sh_1, shrunken endosperm. *Bz* in presence of *C* produces dark color in aleurone and plant; with the recessive, *bz*, color is modified to a bronze shade. *Wx* produces amylose starch in endosperm and pollen grain which stains blue with I-KI solutions; no amylose starch produced by recessive, *wx*, and starch stains red-brown with I-KI solutions. The order of these markers in the short arm of chromosome 9 is: *I* Sh_1 *Bz Wx Ds* centromere.) No *Ac* factor is present in the plants of this stock. Consequently, no breaks at *Ds* occur in these plants. If, for example, the *Ac* constitution of a plant homozygous for *C*, sh_1, *bz* and *wx* is to be determined, a cross to or by the *Ac* tester plant is made. If the plant being tested has no *Ac*, all the kernels on the resulting ear will be colorless, nonshrunken, and *Wx*. If one *Ac* is present, half of the gametes produced by the *C*, sh_1, *bz*, *wx* plant will carry *Ac*. The other half will have no *Ac*. The kernels on the resulting ear will exhibit this gametic ratio for *Ac*. Those that do not have *Ac* will be totally colorless, nonshrunken, and *Wx*. Those that have *Ac* will be variegated. In these kernels, sectors will be present that show the collective recessive phenotype: *C*, sh_1, *bz* and *wx*. These sectors are produced because *Ac* induces breaks at *Ds* in the *I* Sh_1 *Bz Wx Ds* carrying chromosome in some cells during the development of the endosperm. These breaks result in the formation of acentric fragments carrying all of the dominant

markers. The fragment, in each case, is lost to telophase nuclei in subsequent mitoses. Therefore, all cells arising from those in which the dominant markers have been removed will express the collective phenotype *C*, sh_1, *bz* and *wx*. If the plant being tested has two *Ac* factors, located at allelic positions in one homologous pair of chromosomes, all of the kernels resulting from this cross should be variegated. (Usually, however, a small percent of the kernels are nonvariegated either because no *Ac* is present or because of a high dosage action of *Ac* which so delays the time of *Ds* breaks that none occur during the development of the kernel. These conditions arise from transposition or change in state of *Ac* that can occur in a few sporogenous cells of *Ac* carrying plants.) If two *Ac* factors are present and these are nonlinked, a ratio approximating 1 nonvariegated to 3 variegated kernels will appear on the ears resulting from the test cross. If more than two *Ac* factors are present, not only the ratios obtained but also the timing of breaks at *Ds* may be used to detect the number of *Ac* factors that are present. In these cases, however, verification must be obtained by progeny tests.

The type of test outlined above may be used to detect positions of *Ac* when it is located in the short arm of chromosome 9. For example, plants having the constitution *Ac C* Sh_1 *Bz Wx/ac C* sh_1 *bz wx* or ones that are *C* Sh_1 *Bz Ac Wx /C* sh_1 *bz ac wx* have been crossed by *Ac* tester plants that are homozygous for *I*, Sh_1, *Bz*, *wx*, *Ds* and have no *Ac*. The phenotypes exhibited in the sectors of the variegated kernels on the resulting ears, and the frequency of kernels with sectors of a particular type, indicate in each of these cases, the location of *Ac* within the short arm of chromosome 9. Subsequent tests of the progeny arising from the various crossover classes of kernels in each of these two cases, affirm the particular location of *Ac*.

Tests showing that *Ac* controls mutation at $a_1^{m\text{-}4}$ may now be described. Kernels having particular phenotypes were selected from the self-pollinated ear of plant 6110, and from the ears derived from the two crosses described above. In plant 6110, the chromosome 3 factorial constitution was: $a_1^{m\text{-}4}$ Sh_2/a_1 sh_2; the chromosome 9 factorial constitution was: *C Wx/C wx*. The necessity for indicating this latter constitution will become apparent as the tests are described. One *Ac* was present in plant 6110 but it was not linked to the marker in chromosome 9 and appeared not to be linked to those in chromosome 3. Four classes of kernels were selected from the self-pollinated ear: (1) colorless, Sh_2, *Wx*, (2) colorless, Sh_2, *wx*, (3) variegated, Sh_2, *Wx*, and (4) variegated, Sh_2, *wx*. Since the locus of Sh_2 is very close to that of $a_1^{m\text{-}4}$, plants derived from the first two classes of kernels were expected to be either $a_1^{m\text{-}4}$ $Sh_2/a_1^{m\text{-}4}$ Sh_2 or $a_1^{m\text{-}4}$ Sh_2/a_1 sh_2 in constitution and to carry no *Ac* factor. Those derived from types (3) and (4) above were expected to have either of these two constitutions with respect to markers in chromosome 3; they should have, however, either one or two *Ac* factors, and if two, these should be located, in the majority of such plants, at allelic positions in one pair of homologues.

The constitutions with respect to sh_2 in the plants derived from the above

TABLE 1

Constitution of plants of culture 6424 (column 1) and types of crosses (columns 3 to 8) that have revealed these constitutions for each plant.

Constitution of plant	Plant no.	Crosses with plants of constitutions given in column headings					
		Self-pollinated	$a_1\,sh_2/a_1\,sh_2$ No *Ac*	$a_1\,Sh_2/a_1\,sh_2$ No *Ac*	$a_1\,Sh_2/a_1\,Sh_2$ No *Ac*	*Ac* tester plant	a_1/a_1 1 *Ac*
$a_1^{m-4}\,Sh_2/a_1^{m-4}\,Sh_2$; No *Ac* (plants 1 to 5)	1		+	+			+
	2						+
	3		+	+		+	+
	4		+	+			+
	5			+		+	+
$a_1^{m-4}\,Sh_2/a_1^{m-4}\,Sh_2$; 1 *Ac* (plants 6 to 8)	6		+			+	
	7		+			+	
	8		+				
$a_1^{m-4}\,Sh_2/a_1^{m-4}\,Sh_2$; 2 *Ac*	9	+	+	+		+	
$a_1^{m-4}\,Sh_2/a_1\,sh_2$; No *Ac* (plants 10 to 17)	10			+			+
	11			+			+
	12		+	+		+	+
	13			+		+	+
	14			+		+	+
	15			+		+	+
	16		+		+	+	+
	17		+	+		+	+
$a_1^{m-4}\,Sh_2/a_1\,sh_2$; 1 *Ac* (plants 18 to 25)	18		+				
	19	+	+	+		+	
	20	+	+	+	+	+	
	21		+	+		+	
	22		+	+		+	
	23		+			+	
	24		+	+	+	+	
	25		+				
$a_1^{m-4}\,Sh_2/a_1\,sh_2$; 2 *Ac* (plants 26 to 29)	26		+	+		+	+
	27	+	+	+		+	
	28		+	+			
	29		+				

selected kernels were determined by crosses to plants that were either $a_1\ sh_2/a_1\ sh_2$ or $a_1\ Sh_2/a_1\ sh_2$. (Supply of plants of the former constitution was limited due to early death from disease of many of them. In some of the tests it was necessary, therefore, to substitute plants of the latter constitution.) *Ac* constitutions were determined for many of them by crosses with *Ac* tester stocks. Detection of the presence of $a_1^{m\text{-}4}$ in plants derived from the colorless kernels required the introduction of *Ac*. For this purpose, plants that were homozygous for a_1 and that carried one or more *Ac* factors were used in crosses with the plants derived from these kernels. To show that the factor controlling mutation at $a_1^{m\text{-}4}$ is the same as that which controls breaks at *Ds*,

TABLE 2

Ac constitution of gametes of plants of culture 6424 (table 1) determined by crosses to plants of Ac-tester stock. Constitution of tester stock: 1 Ds/1 Ds; A_1/A_1; no Ac.

Ac constitution of plant	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types on resulting ear	
			Colorless (no *Ac*)	Variegated: colored areas in colorless background (*Ac*)
Group I, No *Ac*	3	♂	324	0
	5	♂	128	0
	12	♂	260	0
	14	♂	123	0
	15	♂	11	0
	16	♂	216	0
Group II, 1 *Ac*	6	♂	251	248
	7	♂	252	288
	19	♂	377	397
	20	♀	135	142
	20	♂	206	188
	21	♀♂	151	135
	22	♂	154	128
	23	♀	42	46
	23	♂	145	134
	24	♂	182	191
Group III, 2 *Ac*	9	♂	47	410
	26	♂	15	457
	27	♂	23	434

some plants arising from the colorless, Sh_2, *wx* class of kernels were crossed by plants with the constitution: a_1/a_1; *Wx Ds/wx ds*; 1 *Ac*. The *Ac* factor was not linked to markers in chromosome 9.

The constitutions of 29 plants in the progeny derived from self-pollination of plant 6110 are entered in table 1 along with the tests conducted with each plant that served to indicate its constitution. These plants were grown in culture number 6424. Nine plants in culture 6424 were $a_1^{m\text{-}4}\ Sh_2/a_1^{m\text{-}4}\ Sh_2$. Five of them had no *Ac* (plants 1 to 5, table 1), three had one *Ac* (plants 6 to 8, table 1) and one had two *Ac* factors (plant 9, table 1). Twenty plants in culture 6424 were $a_1^{m\text{-}4}\ Sh_2/a_1\ sh_2$ and among them, eight had no *Ac* (plants 10 to 17), eight had one *Ac* (plants 18 to 25), and four had two *Ac* factors

TABLE 3

Types of kernels appearing on ears resulting from crosses of plants of culture 6424 (table 1) with tester plants having constitutions entered in column 1. Part I: Tester plants have no Ac. Part II: Tester plants have 1 or 2 Ac factors.

Part I

Constitution of tester plants	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types						Totals
			Colored Sh_2	Colored sh_2	Variegated Sh_2	Variegated sh_2	Colorless Sh_2	Colorless sh_2	
a_1sh_2/a_1sh_2 No *Ac*	1	♀C+♂O+	0	0	0	0	230	0	230
	3	♀C+♂O+	0	0	0	0	133	0	133
	3	♀O+♂C+	0	0	0	0	573	0	573
	4	♀O+♂C+	0	0	0	0	298	0	298
Totals, plants 1 to 4			0	0	0	0	1234	0	1234
	6	♀C+♂O+	1	0	132	0	126	0	259
	6	♀O+♂C+	7	0	507	0	554	0	1068
	7	♀C+♂O+	1	0	132	0	151	0	284
	7	♀O+♂C+	15	0	275	0	295	0	585
	8	♀C+♂O+	3	0	168	0	188	0	359
Totals, plants 6 to 8			27	0	1214	0	1314	0	2555
	9	♂O+	26	0	584	0	95	0	705
	12	♀O+♂C+	0	0	0	0	120	125	245
	16	♀C+♂O+	0	0	0	0	131	120	251
	16	♀O+♂C+	0	0	0	0	144	137	281
	17	♀C+♂O+	0	0	0	0	65	55	120
Totals, plants 12 to 17			0	0	0	0	460	437	897
	18	♀C+♂C+	0	0	75	0	73	134	282
	19	♀C+♂O+	3	0	62	0	83	167	315
	19	♀O+♂C+	12	0	199	0	216	375	802
	20	♀C+♂O+	0	0	53	0	58	114	225
	20	♀O+♂C+	12	0	234	0	267	444	957
	21	♀C+♂C+	4	0	86	0	75	168	333
	22	♀C+♂C+	1	0	99	0	79	154	333
	23	♀C+♂C+	1	0	69	0	70	157	297
	23	♀C+♂O+	20	0	179	1	185	333	718
	24	♀O+♂O+	9	0	174	3	210	363	759
	25	♀C+♂O+	1	0	54	0	72	123	250
Totals, plants 18 to 25			63	0	1284	4	1388	2532	5271
	26	♀O+♂O+	1	0	166	1	0	152	320
	26	♀C+♂O+	4	0	140	0	6	141	291
	27	♀O+♂C+	0	0	134	0	35	178	347
	28	♀O+♂C+	0	0	151	0	1	146	298
Totals, plants 26 to 28			5	0	591	1	42	617	1256
	29	♀	0	0	72	0	28	125	225

TABLE 3 (*continued*)

Part I (*continued*)

Constitution of tester plants	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types						Totals
			Colored Sh_2	Colored sh_2	Variegated Sh_2	Variegated sh_2	Colorless Sh_2	Colorless sh_2	
$a_1\,Sh_2/a_1\,sh_2$	1	♂	0	0	0	0	573	0	573
No *Ac*	3	♂	0	0	0	0	934	0	934
	4	♂	0	0	0	0	846	0	846
	5	♂	0	0	0	0	894	0	894
Totals, plants 1 to 5			0	0	0	0	3247	0	3247
	9	♂	22	0	392	0	49	0	463
	10	♂	0	0	0	0	364	106	470
	11	♂	0	0	0	0	731	236	967
	12	♂	0	0	0	0	705	210	915
	13	♂	0	0	0	0	715	234	949
	14	♂	0	0	0	0	644	200	844
	15	♂	0	0	0	0	431	127	558
	17	♂	0	0	0	0	192	86	278
Totals, plants 10 to 17			0	0	0	0	3782	1199	4981
	19	♂	4	0	182	0	385	198	769
	20	♂	8	0	108	0	185	88	389
	21	♂	12	0	205	0	438	224	879
	22	♂	2	0	206	0	525	249	982
	24	♂	6	0	130	1	237	134	508
Totals, plants 19 to 24			32	0	831	1	1770	893	3527
	26	♂	8	0	532	0	294	280	1114
	28	♂	3	0	457	0	243	194	897
Totals, plants 26 and 28			11	0	989	0	537	474	2011
$a_1\,Sh_2/a_1\,Sh_2$	16	♂	0	0	0	0	535	0	535
No *Ac*	20	♂	8	0	132	0	432	0	572
	24	♂	3	0	111	0	347	0	461
Totals, plants 20 and 24			11	0	243	0	779	0	1033
			Part II						
$a_1\,sh_2/a_1\,sh_2$	4	♂	0	0	244	0	243	0	487
1 *Ac*	11	♂	0	0	78	0	75	145	298
	12	♂	0	0	76	0	60	123	259
	13	♂	0	0	125	0	111	268	504
	15	♂	0	0	34	0	35	60	129
	16	♂	0	0	68	0	62	135	265
	17	♂	0	0	54	0	61	149	264
Totals, plants 11 to 17			0	0	435	0	404	880	1719

TABLE 3 (*continued*)

Part II (*continued*)

Constitution of tester plants	Plant no. in culture 6424	Parentage of 6424 plant in cross	Kernel types						Totals
			Colored Sh_2	Colored sh_2	Variegated Sh_2	Variegated sh_2	Colorless Sh_2	Colorless sh_2	
a_1Sh_2/a_1sh_2 1 *Ac*	1	♂	0	0	251	0	210	0	461
	2	♀	0	0	161	0	158	0	319
	3	♂	0	0	387	0	384	0	771
	4	♂	0	0	157	0	157	0	314
	5	♀	0	0	119	0	115	0	234
Totals, plants 1 to 5			0	0	1075	0	1024	0	2099
	10	♀	0	0	117	0	203	113	433
	12	♀	0	0	98	0	184	83	365
	14	♀	0	0	103	0	202	100	405
	15	♂	0	0	122	0	207	122	451
Totals, plants 10 to 15			0	0	440	0	796	418	1654
a_1Sh_2/a_1Sh_2 1 *Ac*	10	♂	0	0	54	0	143	0	197
a_1Sh_2/a_1sh_2 2 *Ac*, non-allelic and non-linked	12	♂	0	0	248[1]	0	228	150	626

[1] 88 of these kernels had relatively few, late occurring mutations (4 *Ac*); 160 had many more mutations that occurred earlier in the development of the kernel (2 *Ac*).

(plants 26 to 29). The plants having no *Ac* factor were derived from the kernels that were colorless. Those carrying *Ac* were derived from the variegated kernels. There were no exceptions.

Direct tests for the presence of *Ac* were conducted with 19 of the 29 plants in culture 6424. The *Ac* tester stock used in tests of 17 of them was homozygous for A_1 and carried *I* and *Ds* (standard location) in each chromosome 9; no *Ac* was present. The types of kernels appearing on the ears when crosses were made to plants in the tester stock are entered in table 2. The nature of this test has been described above but may be summarized here. If all of the kernels are colorless, then the plant being tested has no *Ac*. If half of the kernels are colorless and the other half are variegated (colored areas in a colorless background), one *Ac* factor is present in the plant being tested. If nearly all of the kernels show colored areas in a colorless background, then the plant being tested carries two *Ac* factors. Two of the 19 plants directly tested for the presence of *Ac* (plants 13 and 17) carried *wx* in each chromosome 9. The silks of these two plants received pollen from plants homozygous for a_1 and carrying *C*, *Wx* and *Ds* at its standard location in one chromosome 9 and *C*, *wx* and *ds* in the homologue; no *Ac* was present in these plants. All of the kernels on the resulting ears were colorless; also, in those kernels that were *Wx*, no *wx* sectors were present, indicating the absence of *Ac* in these two plants.

The types of kernels produced on ears resulting from crosses of these 29 plants of culture 6424 to plants homozygous for a_1 are entered in table 3. The a_1 stocks were either homozygous for sh_2, heterozygous for sh_2, or homozygous Sh_2. Table 3 has two parts. In part I are entered the types and frequency of type of kernels appearing on ears derived from crosses of 28 of the 29 plants with plants homozygous for a_1 and having no *Ac* factor. In part II are entered the types and frequency of type of kernels appearing when plants derived from the colorless, Sh_2 classes of kernels in culture 6424 (plants 1 to 5 and 10 to 17) were crossed with plants homozygous for a_1 but carrying an *Ac* factor.

An examination of the data entered in part I of table 3 will reveal that all of the plants having one or two *Ac* factors, as determined by direct tests (Groups II and III, table 2), produced variegated kernels in crosses with plants having no *Ac* factor. Also, germinal mutations were evident. Those plants in culture 6424 with no *Ac* (Group I, table 2), on the other hand, produced only colorless kernels from the same type of cross. When, however,

TABLE 4

Relation of mutation at a_1^{m-4} in chromosome 3 to breaks at Ds in chromosome 9. Constitution of parents: ♀ a_1^{m-4}/a_1^{m-4}; wx/wx, no Ac × ♂ a_1/a_1; Wx Ds/wx ds; 1 Ac.

Parent plants		Kernel types						
		Variegated aleurone		Colorless aleurone		Variegated aleurone	Colorless aleurone	Totals
♀	♂	*Wx*	*wx* areas in *Wx* background	*Wx*	*wx* areas in *Wx* background	*wx*	*wx*	
6424-2	6427B-9	5	61	77	0	102	103	348
6424-3	6427B-8	7	89	101	0	86	105	388
6424-4	6427B-7	6	50	60	0	63	55	234
Totals		18	200	238	0	251	263	970

these same plants (3 and 5; 12 to 17) were crossed with plants homozygous for a_1 but carrying an *Ac* factor, variegated kernels appeared (part II, table 3). In both part I and part II of this table it may be seen that the variegated kernels were present in the expected classes, and in the expected frequency within each class, on the assumption that *Ac* is the factor responsible for the occurrence of mutation at a_1^{m-4}.

Results of the test, recorded in table 4, leave no doubt concerning *Ac*-control of mutation at a_1^{m-4}. It is the same *Ac* factor that is responsible for producing breaks at *Ds*, wherever it may be located, and for controlling mutability at some other loci of known genic action (see table 6). This test was conducted with plants 2, 3 and 4, each of which was homozygous for a_1^{m-4} and Sh_2 in chromosome 3 and for *wx* in chromosome 9. Pollen from plants homozygous for a_1, and carrying *C*, *Wx* and *Ds* (standard location) in one chromosome 9 and *C*, *wx* and *ds* in the homologue and also a single *Ac* factor, not linked with the markers in chromosome 9, was placed on the silks of these three plants. If *Ac* is the factor controlling mutation at a_1^{m-4}, the types of kernels on the resulting ear, and the frequency of each type, may be predicted. In the *wx*

class, two types of kernels should appear. Half of them should be colorless (no *Ac*) and half should show variegation for color in the aleurone layer due to mutations at $a_1^{m\text{-}4}$ (*Ac* present). In the *Wx* class of kernels, three types should appear. Half of the *Wx* kernels should be colorless, and no *wx* sectors should appear within these kernels (no *Ac*). The other half of the *Wx* kernels should be variegated for aleurone color (*Ac* present) and the majority of them should also have sectors showing the *wx* phenotype. This is because *Ds* is located close to *Wx*. Relatively little crossing over occurs between the loci of these two factors. Thus, the majority of the *Wx* class of kernels will have *Ds* in the *Wx*-carrying chromosome; in only a small percentage of them will *Ds* be absent. When *Ac* is present, breaks occur at *Ds* in some of the cells during the development of the kernel. These result in elimination of *Wx*. As a consequence, sectors having the *wx* phenotype will appear in the mature kernels.

Among a total of 970 kernels entered in table 4, 501 were completely colorless and 469 were variegated for aleurone color. Within the colorless class, 263 were *wx* and 238 were *Wx*. In these *Wx* kernels, no sectors showing the *wx* phenotype appeared. Within the variegated class of kernels, 251 were completely *wx* and 218 were *Wx*. Among these *Wx* kernels, on the other hand, sectors exhibiting the *wx* phenotype were present in 200 of them. In only 18 of them were *wx* sectors absent. It is obvious, therefore, that the factor controlling breaks at *Ds*—that is, *Ac*—is also responsible for mutation at $a_1^{m\text{-}4}$. Without *Ac*, neither breaks at *Ds* nor mutations at $a_1^{m\text{-}4}$ will occur. When *Ac* is present, both will occur. With this information in mind, it is possible to interpret readily the data entered in tables 2 and 3 that have not received detailed comment. It is only necessary to indicate the essential correlations that these data reveal.

In direct tests of *Ac* constitutions, table 2, eight plants proved to have one *Ac* factor (plants 6 and 7, and 19 to 24). Plants 6 and 7 were homozygous for Sh_2. Since Sh_2 is very closely linked to $a_1^{m\text{-}4}$, these plants could be expected to be homozygous for $a_1^{m\text{-}4}$. When they are used in crosses to plants homozygous for a_1 and carrying no *Ac*, a ratio of 1 variegated to 1 nonvariegated kernel should appear on the resulting ear. This ratio was obtained, as shown in part I of table 3. Plants 19 to 24 were Sh_2/sh_2. Therefore, they are expected to be: $a_1^{m\text{-}4}\ Sh_2/a_1\ sh_2$. When crossed to plants homozygous for a_1 and having no *Ac*, a ratio of 1 variegated to 3 nonvariegated kernels should appear on the resulting ear. If the tester plant is also homozygous for sh_2, the variegated kernels would be present almost exclusively in the Sh_2 class, and the ratio of kernel type in this class would be 1 variegated to 1 nonvariegated. The ratios obtained from tests of these six plants (part I, table 3) fit this expectancy.

Direct tests of *Ac* constitutions showed that plants 9, 26 and 27 had two *Ac* factors (table 2). Plant 9 was homozygous for Sh_2 and therefore could be expected to be homozygous for $a_1^{m\text{-}4}$. The ratio of variegated to nonvariegated kernels on the ears resulting from crosses to plants homozygous for a_1 and having no *Ac* factor, should closely reflect the gametic ratio of presence and absence of *Ac*. The ratios obtained (part I, table 3), show that this expectation

is fulfilled. (Because some of the gametes produced by these plants will not carry *Ac*, a small percent of the kernels should be colorless, nonvariegated. Also, a few of them may have *Ac* but carry an altered state of a_1^{m-4} that no longer gives visible mutations or gives so few of them that, in individual kernels, none may have occurred. In order to distinguish between these two possibilities, it is necessary to conduct progeny tests with the plants arising from the nonvariegated kernels.) Plants 26 and 27 were Sh_2/sh_2 and therefore could be expected to be heterozygous for a_1^{m-4}. Approximately 1 variegated to 1 nonvariegated kernel should appear in the backcross test to plants homozygous for a_1 but having no *Ac*. If the tester plant is also homozygous for sh_2, the variegated kernels should be confined, with few exceptions, to the Sh_2 class. As the data in table 3 show, this expectation is fulfilled.

Once the fact is established that *Ac* controls mutation at a_1^{m-4} and that this mutable locus responds in the expected manner to increased doses of *Ac*, it is no longer necessary to apply to each plant all of the tests that have been outlined above. Constitutions may be determined by ratios of kernel types in backcross tests. Plants carrying altered states of a_1^{m-4} or changes of *Ac* affecting its state, location, or number, may be identified by such tests. In these latter cases, however, progeny tests often must be conducted to establish with certainty a particular conclusion drawn from the backcross test.

Mutation or change in state of an *Ac*-controlled mutable locus rarely occurs early in the development of a plant, although a few such occurrences have been noted in all examined cases. Most of the changes at the locus take place in the later developmental stages of the sporophytic tissues. It is such late occurring mutations that are responsible for the completely colored kernels recorded in part I of table 3. Such kernels did not appear in the tests recorded in part II of this table. In this part of the table, the a_1^{m-4} carrying plants did not have *Ac*. Thus, no germinal mutations were expected and none were found. The types of germinal mutation occurring in plants having *Ac* resemble in kind those appearing in the variegated kernels. Among them are darkly pigmented kernels and kernels showing various lighter shades of pigmentation.

The results recorded in table 3 suggest that very little crossing over occurs between a_1^{m-4} and Sh_2. Only 6 variegated kernels appeared in the sh_2 class, and 4 of these 6 appeared when pollen from one plant was used, plant 24. Three of them appeared in crosses that were made with a single collection of pollen from this plant. This suggests that mutation rather than crossing over may be responsible for some of the variegated kernels in the sh_2 class.

Similar tests, although less extensive than those outlined above for the progeny derived from self-pollination of plant 6110, were conducted with plants arising from selected kernels resulting from crosses of this plant to plants that were $a_1\ Sh_2/a_1\ sh_2$, and had no *Ac*, and to a plant that was $A_1\ Sh_2/a_1\ sh_2$ and carried one *Ac*. From this latter cross, variegated kernels considered to have received an *Ac* factor from each parent were selected. These exhibited only a few specks of color scattered over the aleurone layer. Tests of 6 plants arising from them showed the presence of two *Ac* factors, but these were not linked

to each other and not linked to the marked factors in chromosome 3. Direct tests for *Ac* were conducted with several of these plants. A gametic ratio of 3 with *Ac* to 1 with no Ac was obtained. The ratio of variegated to nonvariegated kernels obtained when these plants were crossed to ones homozygous for a_1 and having no *Ac* gave the expected ratios: 3 variegated to 1 nonvariegated kernel among those that received a_1^{m-4} (table 5).

The same types of tests that were used in the analysis of a_1^{m-4} were also conducted with plants carrying a_2^{m-3} and a_2^{m-4}. It was soon evident that mutations occurring at a_2^{m-3} are not *Ac*-controlled. At a_2^{m-4}, however, mutation is

TABLE 5

Types and frequency of type of kernels on ears resulting from cross of ♀ a_1/a_1; *no Ac* × ♂ a_1^{m-4} Sh_2/a_1 sh_2; *2 non-linked Ac factors.*

Constitution of ♀ parent with reference to Sh_2 and sh_2	♂ Parent	Kernel types						Totals
		Colored		Variegated		Colorless		
		Sh_2	sh_2	Sh_2	sh_2	Sh_2	sh_2	
sh_2/sh_2	6426A-1	1	0	48	0	13	65	127
	" -5	6	0	214	0	88	318	626
	" B-2	1	0	97	1	22	107	228
Totals		8	0	359	1	123	490	981
Sh_2/sh_2	6426A-1	8	0	112	0	142	87	349
	" -2	29	0	443	0	479	315	1266
	" B-1	9	0	145	0	173	107	434
	" -2	8	0	213	0	252	155	628
	" -3	29	0	380	0	412	234	1055
Totals		83	0	1293	0	1458	898	3732
Sh_2/Sh_2	6426A-1	12	0	153	0	302	0	467
	" -2	26	0	267	0	576	0	869
Totals		38	0	420	0	878	0	1336

Ac-controlled; and a_2^{m-4} responds to *Ac* in a manner comparable to that observed for all other known *Ac*-controlled mutable loci (see table 6). A detailed account of this analysis will not be included here.

DISCUSSION

From examination of instability of genic action at a number of known loci in maize, it is concluded that mutations need not express changes in genes, but may be the result of changes affecting the control of genic action. It can be shown that this control is effected by nongenic agents carried in the chromosomes. Different agents are present, and may be distinguished by specificities they exhibit in their mode of control of the action of genes. It has been learned that the same agent may operate at different loci of known genic action and that different agents may operate at any one locus. These agents, therefore, reflect the presence of "extragenic" systems carried in the chromosomes, which control genic expression. One of them is the *Ds-Ac* two-unit system,

TABLE 6

Known loci (column 1) at which mutability, under the control of Ac, has arisen (column 2) and mutability at the same loci controlled by other systems (column 3). (The figures following the symbols represent the sequence of appearance in the Cold Spring Harbor cultures.)

Symbol of normal, dominant factor at locus	Instability controlled by *Ac*	Instability controlled by system other than *Ac*
C	c^{m-1} c^{m-2} c^{m-4}	c^{m-3}
Sh_1	$sh^{m\,1}$	
Bz	bz^{m-1} bz^{m-2} bz^{m-4}	bz^{m-3}
Wx	wx^{m-1} wx^{m-5} wx^{m-6}	wx^{m-2} wx^{m-3} wx^{m-4}
A_1	$a_{1}{}^{m-3}$ $a_{1}{}^{m-4}$	$a_{1}{}^{m-1}$ $a_{1}{}^{m-2}$
A_2	$a_{2}{}^{m-4}$	$a_{2}{}^{m-1}$ $a_{2}{}^{m-2}$ $a_{2}{}^{m-3}$

[1]For origins of sh^m from *Sh*, see MCCLINTOCK 1952.

whose action at the locus of A_1 has been considered in this report. Detailed summaries of its operation are given elsewhere (McCLINTOCK 1951). Therefore, considerations of the behavior of this system need not be repeated here.

Instability of genic action, under the control of the *Ds-Ac* system, has been examined at six known loci (table 6). At four of them (*C*, *Bz*, A_1 and A_2) genic action is associated with development of anthocyanin pigmentation. At one of them, *Wx*, it is associated with the chemical constitution of starch in endosperm and pollen grain. In the sixth case, Sh_1, it is associated with a morphological structure of the endosperm. At five of these six loci, instability under the control of other systems has appeared in the Cold Spring Harbor cultures, as indicated in the table. There appears to be no relation between the primary type of action of the gene and the type of system that can serve to control it.

Inception of instability at a locus of known genic action is interpreted as the result of the insertion of a specific controlling unit at or adjacent to the locus. Such insertion is an expression of the phenomenon of transposition, which is characteristic of controlling units. Transposition in the case of *Ds* or *Ac* may be detected readily, and its mechanism has been analyzed (McCLINTOCK 1951). It is initiated by chromosome breaks and fusions that occur at the sites of the controlling units; and it results in their removal and insertion at other positions in the chromosome complement.

In this two-unit system, it is the *Ds* component that is responsible, when inserted at the locus of a gene, for modification of the action of the gene; whereas the function of *Ac* is to control the occurrence of changes at *Ds*. Some of the initiating events at *Ds* result in detectable chromosome alterations. Others produce changes that are not microscopically visible and these probably are responsible for most of the observed cases of altered genic expression. Removal of *Ds* from the locus of the gene is one of them. Others may result in modifications of the chromatin material at the locus, either structurally, quantitatively, or by insertion of chromatin from elsewhere. In this case, a particular type of change in genic expression might reflect a particular type or types of change in chromatin components at the locus. Evidence in support of this assumption has been obtained (McCLINTOCK unpublished).

The *Ac* component also undergoes transposition, and its insertion at new positions has been noted; but no cases of direct *Ac* control of genic change have been observed. *Ac* controls the occurrence of changes at *Ds*, wherever *Ds* may be located. Because methods of detecting the presence of *Ac* are both simple and sure, it is possible to determine, in any case of mutability, whether or not it is controlled by the *Ds-Ac* system. Also, because *Ds* may be transposed to various positions in the chromosome complement, inception of *Ac*-controlled mutability at different known loci can be anticipated. It is only necessary to provide adequate means for their detection, such as those outlined in this report.

Examination of many different cases of instability of genic expression has shown that the basic mechanism producing it is alike in all cases. The mutation results from a change occurring at the locus of a gene during a mitotic cycle. The locus in each sister chromatid may be altered, but the modification need not be alike in both. Consequently, genic expression may differ in the two resulting cells and in their progeny. The factor controlling the time of occurrence of changes at the locus of a gene in future cell and plant generations—that is, the *Ac* factor in the case described here—may likewise be altered during mitotic cycles. As a consequence, sister chromatids may differ with respect to this controlling unit. Segregation of sister chromatids at anaphase will give rise to two cells that may differ with regard to this component. In the progeny of these two cells, this difference will be expressed at the locus of the gene whose mutation it controls. A twin sector or a twin spot may appear in the mature tissue. The two components of the sector or spot may show marked differences in mutation time and/or frequency. (A twin sector may be recognized if the mutation affects a phenotypic change that can be expressed in any cell of the tissue, such as mutation from *wx* to *Wx* in the endosperm. A twin spot will appear if the mutation can be expressed only in a restricted part of the tissue, such as anthocyanin pigmentation in the endosperm tissue which is confined to the outermost layer, the aleurone layer. In this latter case, however, the presence of twin spots reflects the presence of twin sectors.) The two sectors of a twin may express differences in various ways: by absence of mutation in one and presence in the other, by changes in mutation time in the sector

showing mutation, or by changes in mutation time in each sector. If, during a single mitosis, a change occurs at the locus of the gene and also at the locus of the controlling unit, the result may be twin sectors that exhibit differences in type of mutation as well as in time of occurrence; that is, they may differ in presence or absence of mutation, in mutational type in each sector, and in time of mutation. In the *Ds-Ac* system, it is known that such coincidences occur with high frequency.

Recently, BRINK and NILAN (1952) and BRINK (1953) have presented evidence, from a study of variegated pericarp in maize, that supports the generalizations stated here and elsewhere (MCCLINTOCK 1950, 1951) concerning the existence and behavior of controlling units. Recognizing the significance of twin spots, they analyzed the progeny of several of them. The examples selected were characterized by mutation to dominant *P* in one spot and change in pattern of variegation in the twin. The time and frequency of occurrence of mutation was much altered in the latter. Examination of progenies derived from spots of the latter type revealed the presence of a mendelizing unit, which BRINK and NILAN call Modulator because its presence is responsible for the altered pattern of mutation. This unit, in the progeny of some spots, was inherited independently of *P*. In the progeny of others, it showed linkage to *P*. A striking similarity to *Ac* is evident in this case. With respect to *Ac* it is already known that plants having one *Ac* factor may produce sectors in which two *Ac* factors are present. In some of them, the two *Ac* units occupy different positions in the chromosome complement: one at the former location and one at a new location, or both at new locations. They may also show independent inheritance in some cases and linked inheritance in others.

Evidence obtained by PETERSON (1953) in a study of mutability at a locus in maize concerned with chlorophyll development (pale green to green) suggests the presence in this case also of a controlling unit, which likewise appears to undergo transposition.

In a discussion of the possible significance of the evidence obtained from studies of genic instability (MCCLINTOCK 1951), it was suggested that controlling units are present in all nuclei, and that they serve to regulate genic action during the development of an organism. Their presence, in the cases of observed genic instability, has been revealed by their displacement. Such displacement, involving units of any one system, might arise in any strain of maize. Detection of a displaced unit would depend upon available methods. In the case of *Ac*, tester stocks have been developed that can reveal its presence. *Dt* (Dotted), a controlling unit somewhat similar to *Ac* in its mode of action, can be detected through the behavior of the recessive a_1; when *Dt* is present, a_1 mutates to higher alleles of A_1. NUFFER (1953) examined a number of strains of maize from various geographic regions in order to determine if *Dt* was present in any of them. It was found in one strain from Brazil and in another strain from Peru. The original *Dt*, discovered by RHOADES, appeared in a strain originating in Mexico; and its location was determined as in or adjacent to the terminal knob of the short arm of chromosome 9. The locus of

Dt in the strain coming from Brazil is not in the chromosome 9 short arm; linkage studies suggest that it is located in chromosome 6. The locus of *Dt* in the Peruvian strain has not yet been determined. All three of these *Dt* factors behave alike in their mode of producing mutation at a_1. Since transposition, often without change in action, characterizes controlling units, it is not surprising that *Dt* units are present in unrelated strains of maize, and that their locations in the chromosome complement differ. As further methods are devised to detect controlling units, it is anticipated that more of them will be discovered.

SUMMARY

Previous studies of the origin and mode of expression of genic instability at a number of known loci in maize led to the following conclusions. Extragenic units, carried in the chromosomes, are responsible for altering genic expression. When one such unit is incorporated at the locus of a gene, it may affect genic action. The altered action is detected as a mutation. Subsequent changes at the locus, initiated by the extragenic unit, again can result in change in genic action; consequently, a new mutation may be recognized. The extragenic units undergo transposition from one location to another in the chromosome complement. It is this mechanism that is responsible for the origin of instability at the locus of a known gene; insertion of an extragenic unit adjacent to it initiates the instability. The extragenic units represent systems in the nucleus that are responsible for controlling the action of genes. They have specificity in that the mode of control of genic action in any one case is a reflection of the particular system in operation at the locus of the gene.

One extragenic system controlling genic expression is composed of two interacting units. It is the so-called Dissociation-Activator (*Ds-Ac*) system. Both *Ds* and *Ac* undergo transposition. The *Ds* component, when inserted at the locus of a gene, is responsible for modification of genic expression. Subsequent changes at the locus, initiated by *Ds*, result in further modification of genic expression. The *Ac* component in this two-unit system controls when the changes at *Ds* will occur. From the conclusions stated above, it was anticipated that the *Ds-Ac* system could operate at any locus of known genic action. This is because the *Ds* unit may be transposed to various locations in the chromosome complement. To obtain this type of instability at any one locus of known genic action, it is only necessary to provide adequate means for its detection. The methods used to obtain and detect this type of instability at the A_1 locus in chromosome 3 and at the A_2 locus in chromosome 5 are described. A detailed analysis of one such case is presented in this report.

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ARTIFICIAL AND NATURAL HYBRIDS IN THE GRAMINEAE, TRIBE HORDEAE. VI. CHROMOSOME PAIRING IN SECALE CEREALE × AGROPYRON INTERMEDIUM AND THE PROBLEM OF GENOME HOMOLOGIES IN THE TRITICINAE

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A LARGE number of hybrids have now been made between cereal grain species of the genera Triticum and Secale on the one hand and various species of wild grasses belonging to the tribe Hordeae on the other. Of particular interest have been those hybrids involving perennial species of the genus Agropyron. The well-known hybrids between Triticum and Agropyron have been studied cytologically by many workers, and are known to exhibit consistently a certain amount of chromosome pairing at meiosis (SAPEHIN 1935; PETO 1936; OSTERGREN 1940; LOVE and SUNESON 1945; MATSUMURA 1949). The presence of this pairing has led these authors to the assumption that chromosomes derived from Agropyron are pairing with those from Triticum, and therefore that homology exists between genomes belonging to these separate genera. The possibility has often been overlooked that, since both parents of the hybrids are themselves polyploids, much or all of the pairing observed in the hybrids may be autosyndesis between chromosomes derived from the same parental species rather than allosyndesis between chromosomes belonging to the different genera.

Hybrids involving Secale provide particularly favorable material for judging whether auto- or allosyndesis is taking place. Secale chromosomes are considerably larger than those of Triticum or Agropyron, and can be easily recognized at meiosis, while those of Triticum and Agropyron cannot be distinguished from each other at this stage. Careful study of meiosis in such hybrids, therefore, should provide critical evidence concerning the amount of autosyndesis which can take place between the different sets of any Agropyron species. From such evidence, the amount of allosyndesis found in Triticum × Agropyron hybrids could be estimated at least to a certain extent.

The only records in the literature known to us of hybrids between Agropyron and Secale are those of VERUSHKIN (1935), LJUBIMOVA (1937), and TSITSIN (1937) on *Secale cereale* × *Agropyron intermedium*, and of CRASNIUK (1935) and FAVORSKY (1935) on *S. cereale* × *A. cristatum*. The cytology of the hybrids concerned was studied by LJUBIMOVA and FAVORSKY. LJUBIMOVA reported 0–5 bivalents in *Secale cereale* × *Agropyron intermedium* ("*A. glaucum*"), which she believed to be due to allosyndesis. In *S. cereale* × *A.*

cristatum, FAVORSKY found 7 bivalents more or less regularly present, and therefore assumed that the chromosomes of *S. cereale* are homologous with 7 of the 14 chromosomes derived from *A. cristatum.* But since MYERS and HILL (1940) found that tetraploid *A. cristatum* has the cytological behavior of an autotetraploid, the pairing observed by FAVORSKY is best explained as autosyndesis, with little or no association between the chromosomes of *A. cristatum* and those of rye. In the present paper, evidence will be presented which strongly supports the same interpretation for the pairing found in the *S. cereale* × *A. intermedium* hybrid.

MATERIAL AND METHODS

The techniques of hybridization, fixation of cytological material, and making aceto-carmine preparations are the same as those previously employed (STEBBINS and TOBGY 1944; STEBBINS *et al.* 1946). In the summer of 1949, sixty florets from one head of cereal rye, var. "Merced" were emasculated and pollinated with spikes of *Agropyron intermedium* culture no. 632. Seeds for this culture were obtained from the Carnegie Institution Laboratory, Stanford, and are descended from a collection received from Bolu Turkey by the U. S. Department of Agriculture under the number F.P.I. 109,219. From this pollination, seven seeds were harvested, and six vigorous F_1 hybrids were obtained in 1950. Cytological fixations were made during the seasons of 1950 and 1951.

MORPHOLOGICAL CHARACTERISTICS OF THE HYBRID

The F_1 hybrid between *Secale cereale* and *Agropyron intermedium* is completely perennial, although it lacks the rhizomes characteristic of *A. intermedium.* In spikelet characters it is far more like Agropyron than Secale, and in fact any systematist not acquainted with its origin would unquestionably call it an unusual form of Agropyron. It differs from *A. intermedium* in the shorter internodes of the rachis of the spike, the slight ciliation along the edges of these internodes, the smaller number of florets per spikelet, the smaller number of nerves on the glumes and lemmas, and the distinct though rather short awns. In addition, the glumes and lemmas are more strongly nerved than they are in *A. intermedium* or any of its relatives. The morphological characteristics of our hybrid agree very well with those reported by LJUBIMOVA (1937).

The hybrid has proved completely sterile, although 30 clonal divisions of it have flowered in the garden for three successive seasons. The first attempt to double the chromosome number by colchicine treatment failed, and this attempt has not been repeated.

CYTOLOGY OF THE HYBRID

Meiotic chromosome behavior in the two parents was the same as has been described by previous authors for the species concerned. Plants of the *S. cereale* parent regularly form 7 bivalents at I metaphase, while in *A. intermedium* there are commonly 21 bivalents. The cytological material available of this

species was not good enough for careful analysis, but in occasional sporocytes quadrivalents as well as univalents were seen. There was some lagging of chromosomes in the second anaphase, and in a small number of sporocytes a single bridge fragment configuration was seen. The tetrads, however, were entirely regular, with no micronuclei evident.

Meiosis in the hybrid was very irregular, but a considerable amount of pairing was observed in every sporocyte (table 1). With a few exceptions, this pairing was autosyndesis of chromosomes derived from Agropyron, and the seven larger Secale chromosomes appeared as unpaired univalents (fig. 2). In two of the fifty cells recorded in table 1, a chromosome of Secale was paired

TABLE 1

Chromosome behavior at metaphase I in 50 sporocytes of Secale cereale × Agropyron intermedium.

	Chromosomes from		Number of cells	Percent
	Agropyron	Secale		
Cells with	10 II + 1 I	7 I	1	2
" "	9 II + 3 I	7 I	1	2
" "	7 II + 7 I	7 I	8	16
" "	1 III + 8 II + 2 I	7 I	5	10
" "	1 III + 7 II + 4 I	7 I	6	12
" "	1 III + 6 II + 6 I	7 I	9	18
" "	1 III + 5 II + 8 I	7 I	9	18
" "	2 III + 6 II + 2 I	AS + 6 I[1]	2	4
" "	2 III + 6 II + 3 I	7 I	4	8
" "	2 III + 5 II + 5 I	7 I	1	2
" "	2 III + 3 II + 9 I	7 I	1	2
" "	3 III + 5 II + 2 I	7 I	1	2
" "	3 III + 5 II + 2 I	1 II + 5 I	1	2
" "	3 III + 4 II + 4 I	7 I	1	2

Number of chromosomes synapsed per nucleus.

Range: 12-21; mean 15.83

Number of chromosomes involved in multiple associations.

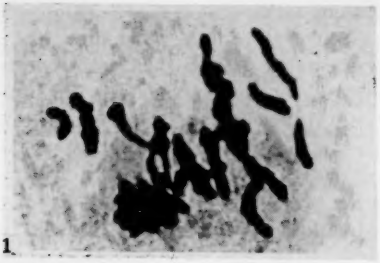
Range: 0-9; mean 3.24

[1]The symbol AS indicates a bivalent composed of one chromosome from Agropyron and one from Secale.

with one from Agropyron by means of a single terminal connection (fig. 1). This same configuration was seen in another cell in which the other configurations could not be fully analyzed. In one sporocyte two of the large chromosomes from Secale were similarly connected with each other (fig. 3).

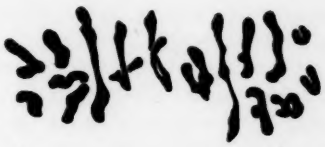
The autosyndesis among the chromosomes derived from *Agropyron intermedium* consisted most often of seven bivalents and seven univalents, or of one trivalent with five or six bivalents and eight or six univalents. Most of the remaining configurations were those involving two or three trivalents, or with nine or ten bivalents. More than eight unpaired Agropyron chromosomes were found in only one cell. The bivalents were mostly of the open type, containing but one chiasma, but closed bivalents were not infrequent.

The later stages of meiosis were all very irregular, and contained many



1

2



3

4

5

FIGURES 1–5.—Meiotic configurations in *Secale cereale* × *Agropyron intermedium*, all × 900. 1. Photograph of I metaphase, showing synapsis of Secale and Agropyron chromosomes. 2 and 3. Drawings of I metaphase; 2, showing seven Secale univalents (at left), two trivalents and five bivalents of Agropyron (center), and five univalents of Agropyron (right); 3, showing five univalents and one bivalent of Secale (left), three trivalents and five bivalents of Agropyron (center), and two univalents of Agropyron (right). 4 and 5. Anaphase, showing lagging chromosomes and bridge fragment configurations.

lagging chromosomes. Bridge-fragment configurations were seen in 34 percent of 82 cells observed at first anaphase (figs. 4, 5). The pollen grains of the hybrid are completely abortive.

DISCUSSION

The most notable feature of this hybrid is the high amount of autosyndesis among the chromosomes derived from *Agropyron intermedium*. The most frequent pairing behavior was that which would be expected if the haploid complement of *A. intermedium* consists of two genomes which are strongly though

not completely homologous with each other, plus a third genome which shows only a slight degree of homology with the other two.

This pairing is considerably greater than that reported by LJUBIMOVA (1937) in the same hybrid, but differences of this magnitude have been found by other authors between different hybrid plants of the same combination. In particular, of the two sister plants of *Triticum durum* var. Mindum × *A. trichophorum* reported by LOVE and SUNESON (1945), one had about the same amount of pairing as the Secale × Agropyron hybrid reported by LJUBIMOVA while the other agreed with the hybrid reported in the present paper.

Even more noteworthy is the fact that the amount of pairing described in this hybrid is very similar to that described in hybrids between hexaploid species of Agropyron and tetraploid species of Triticum. PETO (1936) found in *T. dicoccum* var. Vernal × *A. "glaucum"* (a synonym of *A. intermedium*) a mean number of 14.8 chromosomes associated in bivalents or trivalents, while in *T. durum* var. Mindum × *A. "glaucum"* 13.0 chromosomes were so associated. These values are lower than the mean number of 15.8 chromosomes associated through autosyndesis in the present hybrid. The mean number of associated chromosomes calculated by the present authors from the data of MATSUMURA (1949) on the hybrid *T. turgidum* × *A. "glaucum"* is 15.5, which is slightly higher, but even if this difference is statistically significant it has little or no meaning in relation to genome homologies.

In hybrids between *A. intermedium* (*A. "glaucum"*) and varieties of *T. aestivum* (*"T. vulgare"*) and *T. spelta* the mean number of associated chromosomes is lower. PETO found means of 11.4 and 11.2 associated chromosomes respectively in hybrids with vars. lutescens and Kharkov, while a mean of 12.3 was calculated by the present authors from the data of MATSUMURA on the hybrids with *T. spelta.* In a hybrid involving a Canadian variety of *T. aestivum,* C.A.N. 1835, PETO found a mean of only 2.6 associated chromosomes. The value calculated from MATSUMURA'S data on *T. Timopheevi* × *A. intermedium,* 10.1 associated chromosomes, is somewhat lower than that for most of the other hybrids.

FAVORSKY (1936) reported 7 bivalents, i.e., 14 synapsed chromosomes, as the usual number in *Aegilops speltoides* × *Agropyron intermedium.* This synapsis also is probably autosyndesis of Agropyron chromosomes, and is no indication of affinity between the chromosomes of Aegilops and those of Agropyron. Higher numbers of bivalents were reported in hybrids between *A. intermedium* and *Aegilops variabilis* (n = 14) as well as *Aegilops turcomanica* (n = 21), but these additional bivalents may be chiefly due to autosyndesis among the Aegilops genomes.

Very similar values were found by LOVE and SUNESON (1945), and by POPE and LOVE (1952) on hybrids between wheat and *A. trichophorum,* which is considered a subspecies of *A. intermedium* in the two standard floras of Central Europe, those of ASCHERSON and GRAEBNER, and HEGI. One plant of *T. durum* var. Mindum × *A. trichophorum* had a mean of 14.78 associated chromosomes, while in the other the mean was 3.20. The mean of 10.8 for *T. Timopheevi* × *A. trichophorum* is very close to the mean of 10.1 found

by MATSUMURA in *T. Timopheevi* × *A. intermedium*. Only in *T. macha* × *A. trichophorum* is there a slightly higher mean number of associated chromosomes, namely 18.4. This difference is interesting in view of the lower mean values obtained for hybrids with other species of hexaploid wheat.

In all of these hybrids the type of configurations described and illustrated are very similar to those which result from autosyndesis in *Secale cereale* × *A. intermedium*. There are always relatively few (0–2 or 0–3) closed bivalents, and otherwise the chromosomes are associated by a single chiasma per arm. The number of trivalents is consistently 0–2 or 0–3; quadrivalents are rare or absent, with never more than one per nucleus.

This evidence indicates to the present authors that in all of the F_1 hybrids between *A. intermedium* or *A. trichophorum* and various species of wheat the observed pairing is exclusively or almost entirely autosyndesis between chromosomes derived from Agropyron. The Agropyron chromosomes probably have a few segments in common with those of Triticum, as they also have with those of Secale, but no large degree of homology exists between their genomes as units. The somewhat higher association in the hybrid with *T. macha* probably involves the D (or C in the terminology of PETO and other authors) genome of that species. CHIN and CHWANG (1944) postulated on the basis of chromosome association in hybrids between *T. macha* and other hexaploid as well as tetraploid species of wheat that the D or "C" genome of *T. macha* is somewhat different from that of *T. vulgare* and *T. spelta*, and is perhaps derived from a different species of Aegilops. This genome presumably has several chromosome segments in common with one genome of *Agropyron intermedium* and *A. trichophorum*.

The presence of less autosyndesis among the chromosomes of *A. intermedium* or *A. trichophorum* than that found in the hybrid with *Secale cereale* is probably due, as POPE and LOVE (1952) have suggested, to the presence of genes or disharmonious gene combinations which restrict the association of homologous chromosome segments. This genetically conditioned partial asynapsis is present to some degree in all hybrids with *T. vulgare*, *T. spelta* and *T. Timopheevi*; it is usually absent but may be present in hybrids with the tetraploid emmers.

The tendency of previous authors to underestimate the amount of autosyndesis possible within the haploid complement of *A. intermedium* and *A. trichophorum* is not unnatural in view of the rarity of multivalents in meiosis of the parental species. This fact indicates that there is a great deal of preferential pairing in these species. We must assume, therefore, that the genomes which undergo autosyndesis are by no means completely homologous to each other.

In view of this unexpected amount of autosyndesis in two species of Agropyron, the situation in hybrids between other species of Agropyron and Triticum should be considered. In *T. turgidum* × *A. junceum* OSTERGREN (1940) found most commonly four or five loosely associated rod bivalents, which he attributed to either auto- or allosyndesis. The present evidence would favor the assumption of autosyndesis for all or nearly all of these associations,

in view of the fact that this hybrid is rather difficult to make, and is completely sterile.

By far the highest degree of synapsis occurs in hybrids between various species of Triticum and *Agropyron elongatum*. PETO (1936) found a mean number of 31.3 to 42.4 chromosomes synapsed in *T. dicoccum* var. Vernal × *A. elongatum* and 36.9, 41.8 and 43.0 chromosomes synapsed in three combinations of *T. vulgare* × *A. elongatum*. Since the contribution from *Agropyron elongatum* is only 35 chromosomes, we must assume that some of this synapsis involves chromosomes derived from Triticum. The writers are inclined to believe, however, that this requisite synapsis between Agropyron and Triticum chromosomes is relatively slight, in view of the strong morphological resemblance between *A. elongatum* and *A. intermedium*. A likely assumption is that 28 of the 35 chromosomes in the haploid complement of *A. elongatum* are capable of pairing with each other, while the remaining 7 are partially homologous to one genome of tetraploid and hexaploid Triticum.

The evidence presented in this paper supports in general the viewpoint expressed by many authors (see LOVE and SUNESON 1945) that the relationships of the species of Triticum, Agropyron, and related genera cannot be expressed entirely on the assumption of the existence of a number of separate chromosome sets or genomes which are either completely homologous or completely nonhomologous with each other. Since chromosome pairing depends upon homology betwen segments of chromosomes, as well as upon genetic and environmental factors, rather than upon total homology or nonhomology between chromosome sets, varying degrees of homology and pairing between different genomes can be assumed to be the rule rather than exceptional occurrences. Nevertheless, the relation between the present evidence and previous assumptions about relationships between species of Triticinae can be perhaps most clearly expressed by assigning approximate genome formulae to the species concerned.

According to the conventional system (see SEARS 1948), the gametic formulae of the diploid, tetraploid and hexaploid species of wheat are respectively A, AB and ABD, while the weedy tetraploid species *T. Timopheevi* is AG. The species of Agropyron most nearly related to wheat, *A. elongatum*, has been given the formula AXXYY by PETO (1936) and BEEFF by MATSUMURA (1951). In the opinion of the present authors, the formula of MATSUMURA is more appropriate. It is hard to see on morphological grounds how *A. elongatum* could contain the A genome, which exists in *T. monococcum*, while MCFADDEN and SEARS (1946) have suggested that the B genome of Triticum may be derived from Agropyron. The use of E and F seems more appropriate than X and Y, since they connote genomes not distantly related to those of wheat, and containing a few homologous segments in common with them. However, the existence of partial homologies would be expressed by the present authors by giving *A. elongatum* the formula $B_2E_1E_2F_1F_2$.

The formula of *A. intermedium* (*"A. glaucum"*) is given by PETO as AXY and by MATSUMURA as BEF. In the opinion of the present authors, both of these formulae are incorrect. The evidence presented above indicates no ap-

preciable homology with wheat, but partial autopolyploidy for this species. The general resemblance to *A. elongatum* suggests that the two species have at least one homologous genome in common, but the presence of rhizomes in *A. intermedium* and its similarity in other respects to *A. repens* indicates the presence of at least one genome which is completely unrelated to the E and F genomes of *A. elongatum* or to any of those in Triticum. If this genome were designated N, then the formula of *A. intermedium* (and probably also *A. trichophorum*) would be either E_1E_2N or $E_1N_1N_2$.

Based on previous work of the present authors on diploid species of Agropyron (STEBBINS and PUN 1953), the diploid bearers of some of the genomes involved may be postulated. The E genome is probably borne by the diploid *A. elongatum,* which was described by SIMONET (1935) from southern France, and of which the senior author has grown a culture originating from Ariana, Tunisia. Another diploid species, *A. caespitosum,* also has many morphological characters in common with the decaploid *A. elongatum,* and may bear the F genome. If so, then the F genome has a strong homology to the genome of the American species *Agropyron spicatum,* and this genome has entered into the formation of a large number of Agropyron species of the section Roegneria, as well as into species of Elymus.

These considerations emphasize the fact that no sharp distinction can be made between those species of Agropyron which are closely related to wheat and those which are not. In fact, the writers believe that the interrelationships between the various species concerned would be best understood if no generic distinctions were recognized, and the "genera" Triticum, Aegilops, Agropyron, Secale, Haynaldia and probably Elymus were all merged into a single large genus. It seems unwise, however, to take such a radical taxonomic step until more is known about species relationships, so that this large genus could be subdivided in a meaningful way.

SUMMARY

A hybrid between *Secale cereale* and *Agropyron intermedium* resembles Agropyron in external morphology, and is completely sterile. At meiosis univalents, bivalents and trivalents are formed, nearly all of them resulting from autosyndesis of the Agropyron chromosomes. These can be identified by their size, which is smaller than that of the Secale chromosomes. The amount of autosyndesis in this hybrid is closely similar to the pairing observed by various authors in hybrids between *A. intermedium* ("*A. glaucum*") or *A. trichophorum* and various species of wheat, and ascribed at least in part to pairing between the chromosomes of Agropyron and those of Triticum. In the light of this evidence, the genome formulae of Agropyron species are reviewed, and some revisions are suggested.

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HETEROSIS IN THE PERFORMANCE OF MICE

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HETEROSIS is a subject which has claimed the attention of breeders of plants and animals for three or more decades. Its application has been principally in the improvement of corn, but other economically important crop plants also have been benefited from its use. During the last few years more attention has been given to the employment of heterosis to increase the productivity of animals. Not only do our farmers now grow hybrid corn, but often hybrid swine or hybrid chickens as well.

The feasibility of extensive commercial use of special crosses between highly inbred lines in any species of farm animals depends chiefly upon the size of the advantage in productivity compared with other breeding methods. In addition, the methods best adapted for utilizing inbred lines in crosses will be affected by the rate of reproduction, and by the nature of heterotic effects on performance. Pilot experiments with inbred strains and their crosses in mice can provide much information useful in the planning of genetic experiments with larger animals, since mice, like cattle, sheep and swine are lactating mammals in which the direct maternal influence on survival and early development is highly important. Of course, the applicability of results with mice in work with other species is limited by the similarity in the genetic basis of variation in performance. One important criterion of similarity is the magnitude of inbreeding effects or heterosis in the components of productivity.

Numerous experiments in the crossing of various breeds and strains of laboratory animals have been conducted with rabbits, mice, rats and guinea pigs. The general conclusions are that there are specific genes for size inheritance, but that effects of heterosis, maternal influences and other factors also interact with these genes to affect litter size and growth at various ages. WRIGHT (1922) observed that in crosses between highly inbred strains of guinea pigs there was improvement in every respect over the inbred parent strains. There was marked improvement in the first cross in percentage of young raised and in growth measurements. In the second generation the improvement was in percentage of young born alive, birth weight, number per litter and frequency of litters. The young from crossbred females showed a substantial improvement over the best of the inbred stock, nearly equaling the level of the random bred stock.

GREEN (1931) in reciprocal crosses with mice found the birth weight of the young nearer to the weight of the larger parent strain than to the weight inter-

mediate between the two parent strains. EATON (1941) also found this to be true for the adult weight of crosses between guinea pig strains differing in adult weight. In experiments with mice VETULANI (1931) considered it a case of probable interaction of heterosis and maternal influence. In crossing small females with large males, the effect of heterosis on birth weight counteracts the maternal influence. In the reciprocal cross, the effects of heterosis and maternal influence are cumulative, resulting in a birth weight approximately equal to that of the larger strain.

DICKERSON *et al.* (1946) working with single crosses of Poland China swine showed that crossbred litters exceeded inbreds in litter size, pig weight and litter weight. The latter showed the greatest effect of heterosis. Hybrid vigor was greater for viability than for growth. The crosses were little heavier than inbreds at birth and 21 days, but exceeded inbreds at 56 and 154 days by 12 and 21 percent respectively. At 154 days the crosses exceeded the inbreds in total litter weight by 72 percent. DICKERSON *et al.* (1947) computed the decline in litter size and pig weight due to inbreeding in Duroc and Poland China swine. For each increase of 0.1 in the inbreeding coefficient of the litter there was a decline of 0.2 pigs per litter at birth, 0.4 at 21 days, and 0.5 at 56 and 154 days. In inbred litters, there was no decline in pig weight up to 56 days. At 154 days, however, the decline was 3.6 pounds for each increase of 0.1 in the inbreeding coefficient. The decline was more rapid for Duroc than for Poland China lines. At another station with lines of the same breeds, the decline for each 10 percent increase in inbreeding was only about half as much for litter size at similar ages and almost no decline in pig weight at any age.

HETZER *et al.* (1951) reported similar results with Landrace cross lines. For inbred lines the inbreeding of litters averaged 28 percent and of dams 26 percent, while for crosses the inbreeding averaged 4 and 25 percent, respectively. Crosses exceeded inbreds in number of pigs at birth, 21 and 56 days by 14, 27 and 29 percent respectively. Both prenatal and postnatal viability were higher among crosses. For litter weight crosses exceeded inbreds by 10 percent at birth, 28 percent at 21 days, and 40 percent at weaning. Individual pig weight showed smaller differences. The crosses had slightly higher dressing percentages. These experiments all seem to agree in that viability factors and growth following birth are dependent more on the heterozygosity of the litter than of the dam. Number born alive, their weight at birth and weaning, and the regularity of mating of the dams increase as the heterozygosity of the dams increase.

The present paper reports estimates of the effect of heterozygosity of dam and of progeny on the survival, rate of growth and the prolificacy of mice. They are based on one experiment involving nine inbred lines, three noninbred strains, and all of their possible combinations in linecrosses, topcrosses (random-bred females $\times$ inbred males) and outcrosses, (the reciprocal of topcrosses), as well as on a second experiment including four lines and certain of their crosses and backcrosses.

MATERIAL AND PROCEDURE

The strains of mice used in these experiments were derived from several sources as follows: strains A, B, Br, C, Cd, H, and W from The Roscoe B. Jackson Memorial Laboratory; strains E and S from the Zoology Department of Ohio State University. All of these strains except E and S had been inbred brother to sister for 20 to 45 generations at the time the stock was procured in 1938. Three random-bred control stocks were used; K was obtained from the Pharmacology Division, Food and Drug Administration, and L and M from the Zoological Division, Bureau of Animal Industry, U.S.D.A.

The material reported in this paper includes two experiments. Experiment I was conducted during the years 1938–1942 inclusive. In it, the nine inbred strains were continued by brother-sister matings. The three control strains K, L and M were random bred, care being taken not to mate animals showing common ancestry in a three-generation pedigree. All of the 72 possible cross combinations were made. Outcrosses and topcrosses with the three random bred strains were also made for each of the nine inbred strains, making 27 combinations for each of these types of matings.

Experiment II was carried on during 1946–1947. In this experiment four inbred strains were used. Strain S was the same as in Experiment I. The others were synthesized as follows from Experiment I. A^s, known as the "snowy belly" strain was mainly W strain of the Bureau of Animal Industry stock crossed with a wild mouse, then inbred and selected for the "snowy belly." Possibly six or seven generations of inbreeding had been followed when the stock was used in Experiment II. Cin was inbred from crosses of A and Br of Experiment I and selected for brown-agouti (cinnamon) color. CdE was inbred from crosses of Cd and E of Experiment I; these were dilutes, black-eyed whites and albinos. Inbreeding in Cin and CdE had been followed for at least five generations when the animals were put into the experiment. The four inbred strains were continued by brother-sister matings. The following F_1's and reciprocals were made: $A^s \times$ Cin, $A^s \times$ S, CdE $\times$ Cin and CdE $\times$ S. For the backcross generation F_1 females were mated to males of their maternal parent strain, thus A^s(Cin) females were backcrossed to Cin males. The crisscross or second backcross was produced by mating the first backcross females to males of the original male strain; in the example given, to A^s males.

All strains were kept in the same room and fed the same diet. Data collected included date of birth and sire's and dam's number and strain, number of mice per litter and weight of litter at birth, and weights of individual mice at 15 and 45 days. Age of dam on the date the litter was born was also recorded.

ANALYSIS OF DATA AND RESULTS

Experiment I

General. The number of litters produced by each type of mating varied considerably. Table 1 shows the actual means for the several characters observed

TABLE 1

Unadjusted means for each type of mating, weighting each line or cross equally. Experiment I.

Genetic group	Litter size			Litter weight		Mouse weight		Age of dam
	At birth	At 15 days	At 45 days	At 15 days	At 45 days	At 15 days	At 45 days	
	number	*number*	*number*	*gm*	*gm*	*gm*	*gm*	*days*
9 Inbred strains	6.27	5.60	5.50	36.3	104.3	6.50	18.93	183
72 F_1 combinations	7.27	6.84	6.71	44.9	140.6	6.58	20.96	231
3 Outbred strains	8.10	7.26	7.20	50.8	149.5	6.97	20.35	225
27 Topcross combinations	7.49	6.96	6.75	51.5	144.1	7.46	21.38	220
27 Outcross combinations	7.50	7.06	6.92	46.2	146.2	6.57	21.16	225

in each type of mating. Average litter size and weight per litter and per mouse were lower for the inbred strains than for any of the other mating groups. The average age of dams for inbred litters was also lower, because the females were usually mated first to males of their own strain, in order to assure perpetuation of the line. The advantage of the outbred and topcross litters over the F_1 and outcross litters (from inbred dams) was slight except in weight per mouse at 15 days, which indicated an advantage from greater milk production of the noninbred dams.

Method of analysis. If age of dam has an important influence on performance of the litter, it could bias the estimates of difference between types of matings and magnify the sampling errors of these estimates. For this reason the effect of age of dam was investigated. The partial regressions of each character on age of dam (X_1) and its square (X_2) were calculated, since the age influence was found to be curvilinear (table 2). The regressions on age were

TABLE 2

Partial regression coefficients for each character on age of dam (b_1) and its square (b_2), with degrees of freedom (DF). Experiment I.

Type of mating		Litter size			Litter weight		Mouse weight	
		At birth	At 15 days	At 45 days	At 15 days	At 45 days	At 15 days	At 45 days
Inbred	b_1	.023296	.021129	.021611	.167099	.124237	.001467	.010522
	b_2	−.000057	−.000052	−.000052	−.000388	−.000354	.000001	−.000020
	DF	483	483	483	483	481	2364	2317
F_1	b_1	.017449	.015767	.015340	.113930	.339412	.002168	.004763
	b_2	−.000046	−.000043	−.000042	−.000276	−.000913	.000004	−.000006
	DF	477	477	477	477	474	3233	3164
Outcross	b_1	.013138	.010405	.011214	.075155	.204728	.000795	−.001795
	b_2	−.000041	−.000039	−.000040	−.000258	−.000799	.000001	.000001
	DF	137	137	137	137	137	982	961
Outbred	b_1	.007231	.012343	.011360	.101146	.201218	.001536	−.004078
	b_2	−.000024	−.000038	−.000037	−.000273	−.000713	.000001	.000006
	DF	161	161	161	161	161	1022	1013
Topcross	b_1	.026852	.023095	.022810	.075030	.363370	−.014690	−.020230
	b_2	−.000058	−.000053	−.000053	−.000208	−.000896	.000029	.000038
	DF	186	186	186	186	186	1305	1276

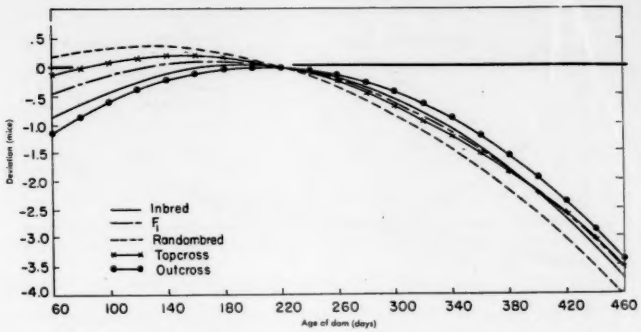

FIGURE 1.—Regression of litter size at 15 days on age of dam expressed as deviation from mean performance for 220-day-old dams in each type of mating.

calculated for each type of mating, based on variation within strains or crosses. The curvilinear regressions of litter size and weight and individual mouse weight at 15 days on age of dam are shown in figures 1 to 3, using the deviations from mean performance for 220-day-old dams within each type of mating to facilitate comparison of types.

It seemed desirable to adjust comparisons between types of mating for differences in age of dam, particularly to minimize error in tests of significance for such comparisons. Since the mean age of dam for all groups except the inbreds fell in the 220–240 day class, 220 days was adopted as the standard age of dam to which means were adjusted. Means for each age of dam subclass of a given type of mating were adjusted to a 220 day age of dam basis by sub-

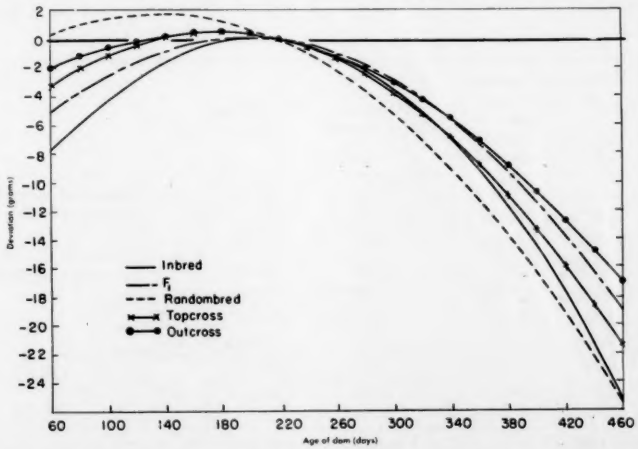

FIGURE 2.—Regression of litter weight at 15 days on age of dam expressed as deviation from mean performance for 220-day-old dams in each type of mating.

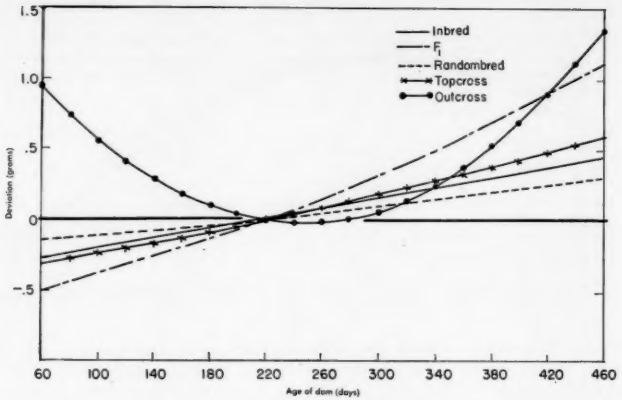

FIGURE 3.—Regression of mouse weight at 15 days on age of dam expressed as deviation from mean performance for 220-day-old dams in each type of mating.

tracting from each the quantity, $b_1(\overline{X}_1 - 220) + b_2(\overline{X}_2 - 48400)$. Weighted averages of these adjusted means were used to obtain the adjusted means for each type of mating. For example, to estimate the difference in litter size at birth between F_1 crosses and inbred parent lines, the means for the inbred and for the F_1 litters from each line of dam were adjusted separately to the 220 day standard age of dam. The mean difference (F_1 – inbred) was then calculated for each of the nine lines of dam. These mean differences were weighted in inverse proportion to the size of their error variances (w), to obtain a weighted mean difference ($\overline{D}$) and to estimate its sampling error, as follows:

Line of dam	Corrected means for litter size at birth		Difference $(F_1 - I) = D$	Weighting (w)	
	F_1	Inbred (I)			
A	6.455	5.864	.591	15.041	n_i = number of inbred litters for a line of dam
B	8.073	6.49	1.584	34.66	
Br	7.99	7.210	.78	18.06	n_c = number of F_1 litters for a line of dam
C	7.95	6.67	1.282	44.950	
Cd	8.542	7.160	1.382	23.59	$w = \frac{n_i n_c}{n_i + n_c}$
E	7.11	6.88	.23	15.94	
H	7.502	5.05	2.456	23.684	$\Sigma(Dw) = 273.69$
S	8.54	7.611	.93	40.162	
W	7.02	6.715	.310	21.500	$D = \frac{\Sigma(Dw)}{\Sigma w} = \frac{273.69}{237.58} = 1.152$
Sum or mean			1.152	237.58	

Significance of the mean difference in litter size born between F_1 and inbred litters was calculated in the following manner:

Source of variation	Degrees of freedom	Sums of squares	Mean square	F
Mean difference ($F_1 - I$)	1	$\frac{(\Sigma Dw)^2}{\Sigma w} = 315.3$	315.3	29.0
Difference × Line of dam	8	$\sum(D^2w) - \frac{(\Sigma Dw)^2}{w} = 86.9$	10.9	P = .0007

The standard error of the weighted mean difference is $\sqrt{\frac{10.868}{237.58}} = .214$. The probability of this large a mean difference in litter size (1.152 mice) due to sampling error is only .0007. In repeated trials of this size, the mean difference would be expected to fall within the range of $1.15 \pm .49$ in 95 percent of

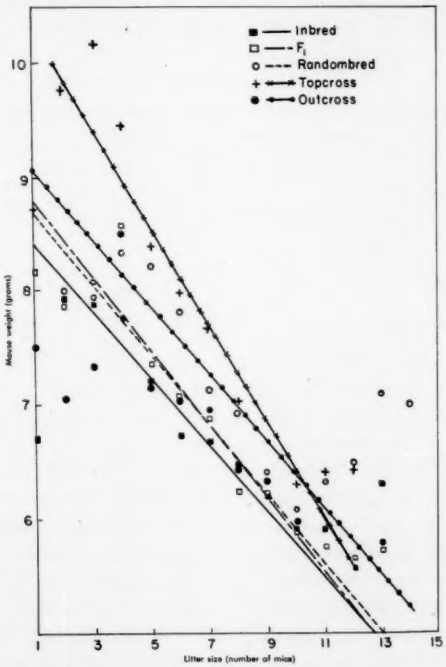

FIGURE 4.—Regression of mouse weight at 15 days on litter size and actual mean mouse weight for each litter size in each type of mating.

the cases. The age-corrected estimates of mean difference for other comparisons between two types of matings were obtained in the same manner for litter sizes and litter weights.

Individual mouse weights are strongly influenced by the number of mice in the litter. In comparisons between types of mating, a difference in weight

per mouse due to a real difference in litter size is an integral part of the effect of type of mating. However, adjustment of mouse weight to a standard litter size basis permits one to estimate the influence that type of mating would have on weight per mouse if litter size were not affected. The actual means of weight for each size of litter and the intra-line or intra-cross linear regression lines are shown in figure 4. Deviations of the plotted means from the linear regression lines include differences between, as well as within, lines or crosses within type of mating. Regressions of 15-day weight per mouse on litter size were very similar for the five types of matings, averaging about −0.3 gram (−5 percent) less weight per mouse for each increase of one in litter size. There is some indication that mice in the very largest litters (11 or more) were handicapped less and those in the very smallest (2 or less) were benefited less than expected from the linear regression. The regression of mouse weight at 45 days on litter size averaged slightly less in grams (−0.26) and much less in proportion to the mean weight (−1.3 percent), compared with those for 15-day weight.

TABLE 3

Partial regression constants for mouse weight on litter size holding X_1 and X_2 constant. Experiment I.

Type of mating	Mouse weight	
	At 15 days	At 45 days
Inbred	−.290153	−.280618
F_1	−.310350	−.157711
Outcross	−.314923	−.227930
Outbred	−.292786	−.287187
Topcross	−.421755	−.381268

The simple regressions of weight on litter size are not entirely independent of the age of dam influences on both litter size and weight per mouse. Hence, to adjust each subclass mean of a type of mating to a standard age of 220 days and litter size of 7, the quantity subtracted was $b_{y1.23}(\overline{X}_1 - 220) + b_{y2.13}(\overline{X}_2 - 48400) + b_{y3.12}(\overline{X}_3 - 7)$, where $\overline{X}_3$ is mean litter size and the b values are the partial regressions on age, its square, and litter size. These revised partial regressions of weight per mouse on litter size are shown in table 3. The adjusted subclass means were then used to obtain the weighted mean difference between any two types of mating and its standard error, as was illustrated above for litter size and litter weight. Numbers of mice rather than of litters were used in computing the weighting for each difference in weight per mouse.

Interpretation of differences between types of matings. Primary interest centered on estimating the influence of heterozygosity of progeny and of dam on the components of productivity. For this purpose certain comparisons between types of mating are more useful than others. The genetic composition of means for the various types of mating may be represented as follows:

Inbreds, $\overline{I} = c$

Linecrosses, $\overline{F}_1 = c + h_1$

Outcrosses, $\overline{O} = c + h_1 + h_2 + g/2$
Topcrosses, $\overline{T} = c + h_1 + h_2 + g/2 + h_3 + m$
Random bred, $\overline{R} = c + h_1 + h_2 + g + h_3 + m - h_4$

where:

c = mean performance of inbred litters of the nine lines

h_1 = effect of greater heterozygosity of F_1 compared with inbred litters

h_2 = effect of possibly greater heterozygosity of $\overline{O}$ or $\overline{T}$ compared with F_1 litters

g = effect of any superiority of $\overline{R}$ over $\overline{I}$ or $\overline{F}_1$ in average effects of genes transmitted

h_3 = effect of greater heterozygosity of dams for $\overline{T}$ and $\overline{R}$ compared with $\overline{I}$, $\overline{F}_1$, or $\overline{O}$ litters

h_4 = effect of any greater heterozygosity of $\overline{O}$ or $\overline{T}$ compared with $\overline{R}$ litters

m = effect of any superiority in maternal influence due to average gene effects for dams of $\overline{T}$ and $\overline{R}$ compared with dams of $\overline{I}$, $\overline{F}_1$ and $\overline{O}$ litters

Estimates for these genetic components were obtained from the appropriate differences between means for types of matings as follows:

$E(h_1) = \overline{F}_1 - \overline{I}$, using weighted average of differences for each line of dam;

$E(h_3 + m) = \overline{T} - \overline{O}$, using weighted mean of differences for each line of sire;

$E(0.5g + h_2) = \overline{O} - \overline{F}_1$, using weighted mean of differences for each line of dam;

$E(0.5g - h_4) = \overline{R} - \overline{T}$, using weighted mean of differences for each line of dam;

$E(g + h_2 - h_4) = (\overline{O} - \overline{F}_1) + (\overline{R} - \overline{T})$;

$E(h_2 + h_4) = (\overline{O} - \overline{F}_1) - (\overline{T} - \overline{R})$.

In comparisons consisting of the sums or differences among four independent means, the proper weight for the complex difference is

$$w = \frac{w_1 w_2}{w_1 + w_2} \quad \text{or} \quad \frac{1}{1/n_1 + 1/n_2 + 1/n_3 + 1/n_4}$$

These estimates of differences are given in table 4, in actual units and as a percentage of the mean for the types of mating compared.

The greater heterozygosity of F_1 compared with inbred litters (h_1 in table 4) has a major influence on the size and weight of litters. Much of the effect on litter size occurs during prenatal development as shown by the advantage of the F_1's over the inbreds of 1.15 mice (16 percent) at birth. This is 7/8 as large as the advantage when the litters are 45 days old. The adjustment for age of dam increased the estimated advantage in litter size for F_1 litters, because inbred litters were produced by dams that were nearer the age of peak reproduction than were dams of the F_1 litters (see table 1 and figure 1). The F_1 advantage in litter weight at 15 days indicates that F_1 mice were slightly smaller than inbred mice from dams of the same age and line, probably due to the increased number of mice suckled. However, the F_1 advantage of 43 grams (33 percent) in litter weight at 45 days of age shows that the F_1 mice had a

TABLE 4

Measures of heterozygosity in mice as affected by heterozygosity of dam, litter and by various other factors, expressed in absolute value (A) and as percentage (P) of the weighted mean of the different types of matings involved. Experiment I.[1]

Source of heterozygosity		Litter size (number mice)[2]						Litter weight (grams)[2]				Mouse weight (grams)[3]			
		Birth	E	15 days	E	45 days	E	15 days	E	45 days	E	15 days	E	45 days	E
$\bar{F}_1 - \bar{I} = h_1$	A	1.15**	.21	1.38**	.22	1.32**	.20	8.4**	1.02	42.9**	4.73	.30**	.07	1.78**	.42
	P	15.8		20.7		20.1		18.6		32.8		4.5		9.2	
$\bar{T} - \bar{O} = h_3 + m$	A	.20	.34	.11	.30	.05	.30	5.2*	2.13	1.9	6.46	.48**	.17	−.18	.17
	P	2.5		1.5		.7		10.1		1.2		6.7		−.9	
$\bar{O} - \bar{F}_1 = .5\ g + h_2$	A	.08	.22	.04	.22	.06	.23	.3	1.25	2.0	4.37	.15	.08	.40*	.14
	P	1.0		.6		.8		.6		1.3		2.2		1.9	
$\bar{R} - \bar{T} = .5\ g - h_4$	A	.21	.22	.10	.19	.18	.22	−.4	1.27	.8	5.60	−.16	.09	−.21	.26
	P	2.6		1.3		2.4		−.7		.5		−2.1		−1.0	
$\bar{T} - \bar{R} + \bar{O} - \bar{F}_1 = h_2 + h_4$	A	−.04	.31	.04	.31	−.07	.35	1.6	1.76	2.9	7.88	.35**	.09	.70*	.27
	P	−.5		.5		−1.0		3.1		1.9		5.0		3.3	
$\bar{O} - \bar{F}_1 + \bar{R} - \bar{T} = g + h_2 - h_4$	A	.35	.34	.20	.30	.33	.31	.1	1.74	4.2	7.36	.01	.15	.19	.32
	P	4.3		2.7		4.5		.1		2.7		.1		.9	

[1]The values expressed in this table and in table 8 are not variances but are mean differences due to increased heterozygosity of one type of mating as compared with another type.

[2]Adjusted to standard age of dam of 220 days.

[3]Adjusted to standard age of dam of 220 days and litter size of 7.

E = Standard error.

**Highly significant p = .01 or less.

*Significant p = .05.

definite advantage in post-weaning growth. When individual mouse weights are adjusted for both age of dam and size of litters, F_1 mice show a small advantage over inbred mice at 15 days (0.30 gm or 4.5 percent) but a much greater advantage at 45 days (1.78 gm or 9.3 percent). The degree of heterozygosity of the young evidently affects survival during embryonic development, as well as to 15 and 45 days, more than the rate of growth of survivors. The estimates of heterosis from F_1 crosses were significant at the .01 or .001 level or less for both litter size and weight per mouse.

The values h_2, h_3 and h_4 as well as g and m cannot be calculated independently for the types of matings which were employed in this group of experiments. Therefore the values shown by the differences between topcrosses, outcrosses and random bred matings represent the combined effects of the respective influences in operation to cause the variations between these types of matings.

The superiority of topcross over outcross litters ($\overline{T} - \overline{O}$) measures the difference in maternal influence arising from the greater heterozygosity and possibly from an advantage in average gene effects, for random bred compared with inbred dams ($h_3 + m$, table 4). Only individual mouse weights and total litter weight at 15 days were significantly better in topcross than in outcross litters (0.48 gm or 6.7 percent for the former and 5.2 gm or 10 percent for the latter). Superior milk production of the more heterozygous random bred dams is presumed to be responsible. It is somewhat surprising to find no advantage in prolificacy or uterine environment. The small advantage of 2.5 percent (0.20 mice) of topcross litters over outcross litters in number of mice at birth may indicate that there is very little difference in the number of ova shed by inbred and by random bred dams. The heterozygosity of litters from topcross and outcross matings should be equal. These facts seem to support the opinion that the difference in litter size at birth between F_1 and inbred lines is due to the greater prenatal survival rates of the heterozygous F_1 litters. However, the random bred dams of the topcross litters may be inherently inferior (i.e., m negative) in prolificacy to the inbred dams of outcross litters, offsetting their advantage in heterozygosity.

Any superiority of outcross over F_1 litters ($\overline{O} - \overline{F}$) could be due either to superiority in average effects of genes from the random bred strains (0.5g) or to greater average heterozygosity (h_2). Only individual mouse weight at 45 days was significantly better for the outcross litters, and this advantage was trivial (0.40 gm or 1.9 percent).

The difference, random bred minus topcross ($\overline{R} - \overline{T}$), provides a second estimate of superiority of random bred strains in average gene effects (0.5g), but in this case less any advantage of the topcrosses in heterozygosity (h_4). Here, topcrosses showed only a small but not significant advantage in weight per mouse at 15 and 45 days, and in litter weight at 15 days.

The difference, ($\overline{O} - \overline{F}_1$) minus ($\overline{R} - \overline{T}$), estimates the sum of the advantages from greater heterozygosity of $\overline{O}$ or $\overline{T}$ litters compared with both $\overline{F}_1$ and $\overline{R}$ litters (i.e., $h_2 + h_4$). A significant advantage, amounting to about 5 percent,

TABLE 5

Unadjusted means for size and weight of litters, mouse weight and age of dam by type of mating, weighting each line or cross equally. Experiment II.

Type of mating	Litter size			Litter weight		Mouse weight		Age of dam
	Birth	15 days	45 days	15 days	45 days	15 days	45 days	
	number	*number*	*number*	*gm*	*gm*	*gm*	*gm*	*days*
4 Inbred lines	6.30	4.82	4.68	32.72	92.72	6.78	19.75	182
8 F_1 combinations	6.91	5.75	5.69	39.42	120.84	6.84	21.16	188
8 Backcross comb.	8.37	7.33	7.23	51.57	148.08	7.04	20.44	163
8 Crisscross comb.	7.90	6.34	6.17	41.17	127.14	6.57	20.71	142

is indicated in weight per mouse at 15 and 3 percent at 45 days (0.35 and 0.70 gm, respectively). There was no effect on viability.

A further test of superiority in transmitted average gene effects for the three random bred strains over the nine inbred lines would be provided by $(\overline{O} - \overline{F}_1) + (\overline{R} - \overline{T}) = g + h_2 - h_4$, if h_2 and h_4 both were positive and similar in size. However, these estimates were small and non-significant for all items of productivity.

Experiment II

General. The actual means for litter size and weight and for mouse weights in Experiment II are given in table 5. Mean age of dams was similar for inbred and F_1 litters (182 and 188 days), but was lower for backcross (163 days) and still lower for crisscross litters (142 days). The unadjusted means indicate important effects of heterozygosity of both dam and litter in viability and growth of young.

Method of analysis. The effects of the considerable differences in age of dam between types of mating were removed by adjusting the mean size and weight

TABLE 6

Partial regression constants for each character on age of dam (b_1) and its square (b_2) with degrees of freedom. Experiment II.

Type of mating		Litter size			Litter weight	
		Birth	15 days	45 days	15 days	45 days
Inbred	b_1	.064441	.053581	.057105	.305373	1.111532
	b_2	−.000157	−.000129	−.000137	−.000728	−.002677
	DF	134	134	134	111	111
F_1	b_1	.002975	−.011762	−.016021	−.085536	−.424478
	b_2	−.000025	−.000004	.000005	.000007	.000285
	DF	184	184	184	161	160
Backcross	b_1	.020860	.024552	.024423	.215616	.549172
	b_2	−.000056	−.000074	−.000073	−.000584	−.001555
	DF	214	214	214	207	206
Crisscross	b_1	−.000801	.003689	.004478	.098381	.246131
	b_2	−.000007	−.000016	−.000020	−.000317	−.000970
	DF	132	132	132	123	123

TABLE 7

Partial regression of mouse weight on litter size holding X_1 and X_2 constant. Experiment II.

Type of mating	Mouse weight	
	At 15 days	At 45 days
Inbred	−.253810	−.213572
F_1	−.209872	−.023336
BX	−.224749	−.173493
CX	−.138892	−.024934

of litters for each line or cross to a standard 170-day basis, using the partial regressions shown in table 6. Individual mouse weight was adjusted for age of dam and difference in litter size by the partial regression of weight per mouse on litter size with age of dam and its square held constant. These regression values are shown in table 7. Differences between the different types of matings were calculated from the adjusted values for litter size and weight and mouse weight as in Experiment I.

Interpretation of differences between types of mating. The genetic composition of the mean for each type of mating in Experiment II may be represented in terms of the same units used in Experiment I, as follows:

$$\text{Inbreds, } \bar{I} = c$$

$$F_1 \text{ Crosses, } \bar{F} = c + h_1$$

$$\text{Backcrosses, } \bar{B} = c + \frac{h_1}{2} + h_3 + \frac{h_1 h_3}{2}$$

$$\text{Crisscross, } \bar{C} = c + 3/4\, h_1 + \frac{h_3}{2} + 3/8\, h_1 h_3$$

The only new symbol is h_1h_3, representing any joint effect of h_1 and h_3, in addition to their independent effects. In Experiment I, this term was included in h_3, since the noninbred dams produced only noninbred litters.

Estimates of the effect of heterozygosity of litter (h_1) and heterozygosity of dam (h_3) were obtained from the following comparison:

(1) $E(h_1) = \bar{F} - \bar{I}$, using weighted mean of the four differences, each for a given pair of reciprocal crosses $\left(\text{e.g., } \frac{(A^s/Cin + Cin/A^s)}{2} - \frac{(A^s + Cin)}{2}, \text{ weighted by } w_1 = \frac{4w_F \cdot w_I}{w_F + w_I} \right)$.

W_I, W_F, W_B, and W_C refer to inbred, F_1, backcross and crisscross matings respectively, and each may be weighted in terms of $\frac{n_1 n_2}{n_1 + n_2}$ where n_1 and n_2 are numbers of observations per line or cross in a given pair of the same type. The proper coefficients are supplied according to the different types of matings used in deriving the estimates of heterozygosity.

(2) $\frac{E(h_3 + h_1h_3)}{2} = \bar{B} - \frac{(\bar{F} + \bar{I})}{2}$, using weighted mean of four differences involving a given pair of lines $\left(\text{e.g., } \frac{A^s(Cin/A^s) + Cin(A^s/Cin)}{2} - \frac{(Cin/A^s + A^s/Cin + A^s + Cin)}{4}, \text{ weighted by } w_2\right)$.

$$W_2 = \frac{16\, W_B \cdot W_F \cdot W_I}{4\, W_F W_I + W_B W_I + W_B W_F}$$

(3) $\frac{E(h_1h_3)}{2} = 2(2\,\bar{C} - \bar{F} - \bar{B})$, using the weighted mean of four differences, each for crosses involving a given pair of lines (e.g., $2\,Cin \times A^s(Cin/A^s) + 2\,A^s \times Cin(A^s/Cin) - (A^s/Cin + Cin/A^s) - (A^s(Cin/A^s) + Cin(A^s/Cin)$, weighted by w_3).

$$W_3 = \frac{\frac{W_C}{4} \cdot W_F \cdot W_B}{W_F W_B + \frac{W_C W_B}{4} + \frac{W_C W_F}{4}}$$

(4) $E(h_2) = \frac{E(h_3 + h_1h_3)}{2} - \frac{E(h_1h_3)}{2} = 3\,\bar{B} + 3/2\,\bar{F} - 4\,\bar{C} - \frac{\bar{I}}{2}$, using a weighted mean of the four differences, each for a given pair of lines $\left(\text{e.g., } \frac{3\,A^s(Cin/A^s) + Cin(A^s/Cin)}{2} + \frac{3\,(Cin/A^s + A^s/Cin)}{4} - \frac{(Cin + A^s)}{4} - 2\,Cin \times A^s(Cin/A^s) - 2\,A^s \times Cin(A^s/Cin), \text{ weighted by } w_4\right)$.

$$W_4 = \frac{\frac{4\,W_B}{9} \cdot \frac{16\,W_F}{9} \cdot 16\,W_I \cdot \frac{W_C}{4}}{\left(\frac{16\,W_F}{9} \cdot 16\,W_I \cdot \frac{W_C}{4}\right) + \left(\frac{4\,W_B}{9} \cdot 16\,W_I \cdot \frac{W_C}{4}\right) + \left(\frac{4\,W_B}{9} \cdot \frac{16\,W_F}{9} \cdot \frac{W_C}{4}\right) + \left(\frac{4\,W_B}{9} \cdot \frac{16\,W_F}{9} \cdot 16\,W_I\right)}$$

In comparison (3), the test of significance for the weighted mean difference is obtained by the F-ratio from:

Source	Degrees of freedom	Sums of squares
Mean difference	1	$\frac{(\Sigma wD)^2}{w}$
Error	3	$\sum (wD^2) - \frac{(\Sigma wD)^2}{w}$

However, in comparisons (1), (2) and (4), the mean of each inbred line is represented in two (a and b) of the four differences. This necessitates using, in place of $\frac{(\Sigma wD)^2}{\Sigma w}$,

$$\frac{(\Sigma w_1 D)^2}{\sum_4 \left(\frac{1}{W_F}\right) w_1^2 + \sum_4 \left(\frac{1}{4\,n_I}\right) \cdot (w_{1a} + w_{1b})^2} \text{ in comparison (1),}$$

$$\frac{(\Sigma w_2 D)^2}{\sum_4 \left(\frac{1}{w_B} + \frac{1}{w_F}\right) w_2^2 + \sum_4 \left(\frac{1}{16\,n_I}\right) (w_{2a} + w_{2b})^2} \text{ in comparison (2), and}$$

$$\frac{(\Sigma w_4 D)^2}{\sum_4 \left(\frac{1}{w_B} + \frac{1}{W_F} + \frac{1}{w_C}\right) w_4^2 + \sum_4 \left(\frac{1}{16\,n_I}\right) (w_{4a} + w_{4b})^2} \text{ in comparison (4).}$$

The expression $\sum_4$ signifies summing 4 quantities represented by the 4 inbred lines. A sample calculation for comparison (1) follows:

Group	n_I	$4\,n_I$	w_1	$w_{1a} + w_{1b}$	w_F	$\frac{(w_{1a} + w_{1b})^2}{4\,n_I}$	w_1^2/w_F
A^s	50	200	28.292	28.292 + 23.794	44.880	13.565	17.835
Cin	31	124	23.794	28.292 + 27.439	37.579	25.048	15.066
CdE	33	132	27.439	27.439 + 25.143	48.069	20.946	15.663
S	24	96	25.143	23.794 + 25.143	45.913	24.946	13.769
						84.505	62.333
						146.838	

$\Sigma w_1 D^2 = 15.719$
$(w_1 D)^2 = 389.066$

Source of variance	DF	Sum of squares	Mean square	F
Mean difference	1	$\frac{389.066}{146.838} = 2.650$	2.650	.608
Difference × cross (error)	3	$15.719 - 2.650 = 13.070$	4.357	

The adjusted mean differences between types of mating are given in table 8.

The effects of heterozygosity of litter (h_1) on litter size and weight per mouse in Experiment II are not as large as in Experiment I, and in no case significant. The heterozygosity of the dams (h_3), however, had a very large and significant effect on nearly all characters observed, the only exceptions being litter size at birth and mouse weight at 45 days.

The interaction of h_1 and h_3 was negative for all characters observed, and significant for litter weight and mouse weight at 15 days.

TABLE 8

Measures of heterozygosity in mice as affected by heterozygosity of dam, litter and their joint effect, expressed in absolute value (A) and as percentage (P) of the weighted mean of the different types of matings involved. Experiment II.[1]

Source of heterozygosity		Litter size (number mice)[2]						Litter weight (grams)[2]				Mouse weight (grams)[3]			
		Birth	E	15 days	E	45 days	E	15 days	E	45 days	E	15 days	E	45 days	E
$\bar{F}_1 - \bar{I} = h_1$	A	.19	.24	.61	.30	.61	.31	3.6	1.4	18.8	7.7	−.07	.24	1.66	.66
	P	2.6		10.6		10.8		8.1		14.1		1.0		8.2	
$\bar{B} - \frac{\bar{F}_1 + \bar{I}}{2} = h_3 + .5\, h_1 h_3$	A	1.50*	.35	1.95*	.46	1.96*	.49	11.7*	3.3	29.5	10.4	.63	.22	.47	.25
	P	19.5		30.1		30.9		23.8		20.2		9.0		2.3	
$2(2\bar{C} - \bar{F}_1 - \bar{B}) = .5\, h_1 h_3$	A	−.12	1.04	−1.69	1.00	−1.82	1.10	−21.8**	2.8	−51.8	18.4	−1.40*	.26	−2.34	1.41
	P	1.5		24.8		27.2		42.9		33.6		20.0		11.0	
$3\bar{B} + \frac{3\bar{F}_1}{2} - 4\bar{C} - \frac{\bar{I}}{2} = h_3$	A	1.63	1.12	3.69*	.96	3.86*	1.01	33.6**	2.5	84.4**	14.1	1.98*	.38	2.86	1.55
	P	20.9		56.8		60.4		68.6		57.1		28.4		13.7	

[1]See footnote 1 under table 4.
[2]Adjusted to standard age of dam of 170 days.
[3]Adjusted to standard age of dam of 170 days and litter size of 7.
Other markings and symbols as in table 4.

DISCUSSION

It will be observed from tables 4 and 8 that there are discrepancies in the values and significance of h_1 and h_3 for the various factors concerned. In Experiment I the heterozygosity of the litter had a highly important effect on litter size, litter weight and individual mouse weight. Inbred mothers with outbred young recorded higher performance than inbred mothers with inbred young. This indicates that the heterozygosity of the litter contributed to higher survival rates, both pre- and post-natal. WRIGHT (1922) calculated that these factors were dependent 75 percent on the breeding of the young. Also, it indicates factors more favorable to growth, even beyond the age of weaning. The only significant effect of the dam's heterozygosity was in litter weight and mouse weight at 15 days. In Experiment II the dam's heterozygosity had an important effect on number of mice at birth and on survival of the mice (litter size) to 15 and 45 days. A marked effect was shown in all characters of litters from crossbred dams in both experiments as with guinea pigs (WRIGHT 1922), but improvement in survival and growth due to heterozygosity of the litter was not apparent in Experiment II. The reason for this apparent discrepancy in the results of these two experiments is not clear. The fact that the inbred strains of Experiment II had been inbred for only 6 or 7 generations in comparison to 40 or more generations for Experiment I can hardly explain the situation. According to WRIGHT (1921) the percentage of homozygosity after 6 generations of brother × sister mating should be 86.7. The remaining heterozygosity could hardly account for as widely varying results as was found between the two experiments.

It is possible, however, that year influence may affect the results. The data in Experiment I were collected during the years 1938–42 inclusive; those of Experiment II in 1946–47. Seasonal comparisons cannot well be made between the two experiments, since S among the inbred lines and K and L of the random bred group were the only strains observed in both periods. Even these were not existent in 1947. Averages for the different years show that strain S showed a continued decline in all the factors considered, except mouse weight at 15 and 45 days, through the entire five-year period covered by Experiment I, but did better in 1946. This year's average, however, was lower than the six-year average. For strain K there was a decline also in 1946 from the previous years. Strain L was fairly constant throughout the period of the two experiments and showed the lowest coefficients of variability for the factors concerned, averaging 9 to 14 percent for litter size and litter weight at various ages, and 4 and 2 percent for individual mouse weight at 15 and 45 days respectively. Corresponding coefficients of variation for S and K ranged from 14 to 32 for litter size and weight at various ages, and were 7 and 2 percent for mouse weight at 15 and 45 days.

From these facts it appears that season had an appreciable effect on survival of the mice mainly to 15 and 45 days, but had little effect on individual mouse weight at these ages. The magnitude of seasonal effect differed in the strains observed.

It is possible that the specific combining ability between the lines used in Experiment II was not as favorable as in Experiment I, and that any effects of heterosis were counteracted by unfavorable gene combinations.

The effect of heterozygosity of dams on mouse weight at weaning (15 days) was apparently due to greater milking ability of the heterozygous dams. FALCONER (1947) made use of the performance of mice to study milk production of the dams. The lactation of the mothers was impaired in proportion to the degree of inbreeding and acted on the young by reducing their growth rate. The effect of heterozygosity of the dams is shown in the present experiments in tables 4 and 8. In table 4, $h_3 + m$, which measures the greater heterozygosity of the dams used in the topcross matings compared with the inbred dams used in the outcross matings plus maternal influences, was significant for litter weight and for individual mouse weight at 15 days. In table 8, h_3 alone measures the difference due to heterozygosity of the dams used in the backcross and crisscross matings compared with the inbred dams of F_1 and inbred litters. The difference was significant for litter weight and mouse weight at 15 days, and also carried over into litter weight at 45 days, probably through the effect of other factors not accounted for.

That the full effect of heterozygosity of the litter is not manifested at the earlier ages is shown by the fact that a significant effect on growth was still apparent at 45 days (table 4) in the superiority of F_1 over inbred litters, of outcross litters over F_1 litters ($g/2 - h_2$), and of outcross and topcross litters over F_1 and random bred litters ($h_2 + h_4$). EATON (1941a, 1941b) found this to be true for both guinea pig and mouse crosses where the effects of heterozygosity carried over to mature weights. DICKERSON *et al.* (1946) also noted this in swine weights at 154 days.

The superiority in litter size at birth and at weaning of F_1 litters over inbred litters in Experiment I agrees well in percentage with the results of HETZER *et al.* (1951) with swine. At birth the advantage was 15 and 14, and at weaning 20 and 29 percent for mice and swine respectively. Advantage in litter weight at weaning also was comparable, 33 percent for mice and 40 percent for swine. The percentage effects on individual mouse weight, as with swine, were less. The highly significant advantage in litter weight from heterozygous dams in experiment II was undoubtedly due to the greatly increased viability of young from heterozygous dams. That the degree of inbreeding of dam affects the performance of the litters, as reported by DICKERSON (1946, 1947) and HETZER (1951), is also shown by the lower averages for the crisscross combinations than for the backcross combinations. The dams of the F_1 litters are inbred, and any advantage of the litters over purely inbred matings is due to the heterozygosity of the litter. Since the dams of the backcross litters are heterozygous, the advantage over the F_1 litters is considerable. The crisscross matings in this experiment, being produced by a backcross to one of the original parent strains, are more inbred than the F_1 dams which produced the backcross litters. Therefore, the performance of the crisscross litters is better than that of the F_1, but not as good as that of the backcross litters.

While the main emphasis in this paper is on heterosis, it may be noted that in comparing these experiments with those of GREEN (1931) and VETULANI (1931), that heredity of the dam is an important factor. In the F_1 and outcross experiments where the dams were inbred, the dams from strains with the heaviest weight at 15 days produced crossbred mice whose weight exceeded that from dams of lighter strains. In many cases the weight rank of the crossbred young followed the weight rank of the inbred parent strains, both for the 15- and 45-day weights.

This may be an indication of maternal influence which has been designated by m in this paper. Its magnitude may not be large or important as table 4 would suggest, but still may be great enough to produce the effect suggested. The small, and for the most part, not significant values of the combinations in which g, h_2 and h_4 appear, suggest that these factors are of little importance. The only significant values where these factors appear are mouse weight at 45 days for $0.5g + h_2$ and 15- and 45-day mouse weight for $h_2 + h_4$. The significance is presumably due to the effect of h_2 since, significant values appear only in the equations where h_2 is present and not in those containing h_4 or 0.5g, except where 0.5g occurs with h_2. This should indicate, then, that outbred and topcross litters are probably more heterozygous than F_1 litters, and that this increased heterozygosity must come through the random bred parent. The effect of this greater heterozygosity is manifested in growth of individual mice probably between 15 and 45 days. There also may be a slight effect on earlier growth.

The heterozygosity of outcross and topcross litters appears to be only slightly greater than that of random bred litters (h_4). The average effects of genes transmitted (g) appears to be only slightly superior in the random bred strains to those of the inbred or F_1 strains.

SUMMARY AND CONCLUSIONS

Two experiments are described in which various types of matings were made between inbred and outbred strains of mice. The types of matings produced in addition to the inbred and outbred or random bred strains were F_1, topcross, outcross, backcross and crisscross matings.

Methods are given for estimating the influence on litter size and on growth, as indicated by weight at different ages, of heterozygosity of dam and of litter, of interaction between these factors, and of minor factors.

The following observations and conclusions may be drawn from these experiments:

1. Age of dam is an important factor in the performance of mice.

2. Noninbred dams reached their maximum litter size production at a younger age than inbred dams.

3. Declining litter size of older dams may account for the continued rise in mouse weights at 15 days with age of dams beyond the age of maximum litter size.

4. Individual mouse weights are strongly influenced by the number of mice in the litter.

5. Comparisons between different types of matings showed the F_1 and outcross mice (dams inbred) to exceed inbreds in performance as measured by litter size at birth, 15 and 45 days, and litter weight and mouse weight at 15 and 45 days. This was an effect of heterozygosity of the litter (h_1). In crosses between unrelated strains which had been inbred for only six generations, the effects of heterozygosity of litters were much reduced.

6. Outbred strains exceeded all other types of matings in performance. This evidently is a result of greater heterozygosity of both litters and parents. Perhaps inbreeding destroyed genetic equilibrium or balance in a way that could not be restored by simply crossing lines in all combinations. However, the difference may be due simply to differences in the foundation stocks of the outbred and inbred strains.

7. Degree of inbreeding of dams influenced performance as shown by comparisons between F_1, backcross and crisscross matings, in which the backcross and crisscross were made to the parent strains composing the F_1 matings. Progeny of the more heterozygous dams (F_1 used in the backcross matings) exceeded in performance those from less heterozygous dams. Topcross and outbred matings also showed the same effect but to a much lesser degree.

8. Heterozygosity of dams, plus superior maternal influence ($h_3 + m$) due to average gene effects on dams of topcross and outbred litters as compared to dams of inbred, F_1 and outcross litters, had a significant influence on litter weight and on mouse weight at 15 days. This may be manifested through greater milking performance of the dams.

9. The effect of greater heterozygosity of outcross or topcross litters compared with F_1 (h_2) and with random bred (h_4) litters was very small and significant only for mouse weight at 15 and at 45 days.

10. Effect of superiority of random bred over inbred or F_1 in average gene effects (g) transmitted was negligible.

11. Full effects of heterosis are not always manifested early, as effects of h_1 and h_2 and of $h_2 + h_4$ were larger at 45 days than at 15 days for mouse weight.

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THE INFLUENCE OF MINUTES UPON SOMATIC CROSSING OVER IN DROSOPHILA MELANOGASTER[1]

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THE Minutes are a group of dominant mutants, homozygous lethal, located on all four chromosomes of *Drosophila melanogaster*. Wherever located, they produce the same general physiological conditions resulting in reduced viability and lengthened larval life. STERN (1936) studied the mechanism of mosaic formation under the influence of certain Minutes and demonstrated that the mosaicism is a result of somatic crossing over. Apparently, the Minute physiology is able to influence chromosomal behavior resulting in an increased frequency of crossing over in somatic cells. Moreover, some interesting relationships between the locus of the Minute and the region of crossing over were shown to exist. While spots of sex-linked characters occurred with higher frequency when either sex-linked or autosomal Minutes were present than in non-Minute flies, sex-linked Minutes were more effective than autosomal ones. Third chromosome Minutes were capable of influencing crossing over in the third chromosome, while sex-linked Minutes were markedly less effective.

Furthermore, in the third chromosome studies an arm to arm correspondence was shown to exist: right arm Minutes more strongly influenced right arm crossing over than did left arm Minutes and the latter, in turn, were more effective in influencing the behavior of the left arm than were the right arm Minutes.

These findings have posed some interesting questions. Is the behavior of the third chromosome in the presence of a third chromosome Minute an isolated, specific phenomenon or is it a generalized expression of the Minute physiology? Do the relationships found to exist between the third and first chromosome apply also to the second and first chromosome? Accordingly, a series of experiments was undertaken to test the behavior of the first and second chromosomes under an experimental set-up similar to that of STERN's work.

THE EFFECT OF SECOND CHROMOSOME MINUTES UPON THE BEHAVIOR OF THE SECOND CHROMOSOME

Choice of materials

It was originally intended to use two left arm and two right arm Minutes for this work. However, $M(2)S^7$, a right arm Minute, was mistakenly used twice, once for itself and a second time in place of a selected left arm Minute

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with the result that the effects of two right arm and one left arm Minute comprise this study. The two MS^7 series are designated MS^7A and MS^7B. The other two Minutes chosen were $M(2)z$ on the left arm, and $M(2)l^2$ on the right. They are located as shown below. (Loci from BRIDGES and BREHME 1944.)

S	*Mz*	*Bl*	*cn*	MS^7	Ml^2	*bw*
1.3	*12.9*	*54.8*	*57.5*	*77.5*	*101.2*	*104.5*

K
55

Each of the three Minutes was combined in a chromosome with several mutant genes in such a way that crossing over would result in phenotypically detectable spots. Mutants affecting eye color and bristle structure were selected since they seemed to provide the most felicitous phenotypic effects and, at the same time, to utilize large surface areas. Star, Bristle, cinnabar and brown were chosen as markers. Their loci are indicated in the previously mentioned figure.

Before combining the Minute with the marker genes into the desired experimental and control genotypes, the second-chromosomal material of each Minute and marker stock was replaced on either side of the Minute or marker gene by Canton-S chromosomal material. For example, the chromosome to the right and to the left of the MS^7 locus was replaced, in a series of crosses, with Canton-S material. Similar replacements were made with each of the other Minutes. By this process, which may be referred to as cantonization, the second chromosomes of the experimental and control flies were made identical except for the presence, in the experimentals, of certain Minutes. It is to be noted, however, that there were small, specific regions on either side of each Minute and marker locus concerning which it was impossible to determine whether they had been replaced by Canton material.

Following is a diagrammatic representation of the three experimental chromosomes:

S	*Mz*	*Bl*	*cn*
1.3	*12.9*	*54.8*	*57.5*

S	*Bl*	*cn*	MS^7
			77.5

S	*Bl*	*cn*	Ml^2
			101.2

These chromosomes were maintained in stocks in which they were balanced over the *Cy* al^2 lt^3 sp^2 inversion complex.

For experimental purposes flies containing the above chromosomes were crossed to homozygous cinnabar brown flies thus deriving genotypes which may be represented as follows for the left and right arm Minutes respectively. Phenotypically, each fly was Minute, Bristle and cinnabar.

S	*M*	*Bl*	*cn*	+
+	+	+	*cn*	*bw*

S	*Bl*	*cn*	*M*	+
+	+	*cn*	+	*bw*

A stock, *S Bl cn/Cy,* was made from the cantonized *S, Bl,* and *cn* stocks, balanced over the Curly complex. These flies, in turn, were crossed to homozygous *cn bw* flies to derive the genotype *S Bl cn* +/+ + *cn bw,* which served as a control in this study.

It was found, as the work progressed, that the Star mutant used produced such phenotypically variable results that no attempt was made to identify non-Star tissue present within a genotypically Star eye. However, it was decided not to remove this mutant from the stocks but to leave it as a common element in the genotypic background.

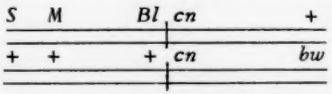
Crosses were made reciprocally and the flies were raised in half-pint bottles at 25°C on a standard agar, corn meal and molasses food mixture.

Analysis of results

STERN (1936) demonstrated that the presence of mosaics under his experimental conditions could be explained on the basis of crossovers involving two strands of a four-strand group. During the mitotic prophase of Diptera, homologous chromosomes are paired and each homologue " splits " into two sister chromatids (METZ 1916). The result is a tetrad-like structure as in meiosis. Indeed the resemblance that this cytological picture presents to meiosis is extraordinary, inasmuch as chiasmata are formed by the large autosomes of males and females and by the X's of females in neuroblast tissue (KAUFMAN 1934). However, in the mitotic anaphase the kinetochores divide and division is equational in relation to the kinetochores. Crossovers occurring between the kinetochore and a given locus heterozygous for a pair of alleles, followed by segregation, result in two cells homozygous for one or the other of the alleles. Tissues developing from such cells with newly acquired genotypes may be phenotypically different from tissues surrounding them and will, thus, be detectable. (It is to be noted that Star, Bristle and the Minutes are lethal when homozygous.)

The choice of the experimental genotypes stems from these considerations. Their significance will be discussed in the following paragraphs.

Mz. The accompanying diagram schematically represents the prophase chromosomes in the case of the left arm Minute.



The type of mosaic produced depends upon the region of crossing over. Exchanges along the right arm anywhere between the kinetochore and *bw* manifest themselves in the appearance of white patches in an otherwise cinnabar eye. Bristle mosaics cannot result from right arm crossovers. In the case of left arm crossovers, no eye mosaics may be produced. Two types of bristle mosaics, on the other hand, may be expected as a result of left arm crossovers. If the crossover lies in the region between the kinetochore and *Bl* then non-*Bl,*

non-*M* mosaics are the result. Crossing over between the *Bl* and Minute loci produces bristle mosaics that are *Bl*, non-*M*. Only the main bristles of the thorax and head were observed in the work concerned with the second chromosome.

It is reasonable to expect that double crossovers might have occurred. There are several instances where the phenotype of the mosaic is explained only by the occurrence of a double crossover. These cases are very rare, however, and are characterized by having one region in the right arm and the second in the left. It has, therefore, been assumed throughout that any given mosaic is the result of a single crossover except where single crossing over cannot account for the appearance of a specific mosaic. For example, wherever patches of white eye tissues have appeared in the *Mz* flies, it has been assumed that they are the result of a single crossover to the right of the kinetochore although the same result is possible following a specific three-strand double crossover. It may be added that in the case of the left arm Minute, a double

TABLE 1

Number and distribution of crossovers in the case of the left arm Minute.

Number of:	Mz			Control		
	Male	Female	Total	Male	Female	Total
Right arm c/o	1	2	3	0	2	2
Left arm c/o						
Between *Bl* & k	12	3	15	0	0	0
Between *M* & *Bl*	4	2	6			
Total c/o	17	7	24	0	2	2
			(0.49%)			(0.065%)
Total flies inspected	2479	2397	4876	1431	1630	3061

crossover with one region between the kinetochore and *Bl* and the other between *Bl* and *M* would result in *M*, non-*Bl* spots. This type of mosaic was not found.

Had double crossovers involving the two arms of the second chromosome occurred, one might have expected instances where an eye mosaic was associated with a bristle mosaic. However, no such cases were found. (The bristle genotypes cannot be differentiated on the basis of the hairs of the ommatidia, so that identification of double crossing over involving an eye mosaic depends upon the appearance of a spot at the edge of the eye and overlapping the surrounding tissue.) The absence of such critical types of mosaics may serve as a measure of the very low frequency of double crossovers.

Table 1 summarizes the experimental data pertaining to the number and regions of somatic crossing over.

Among 3061 control flies, 2 occurrences of somatic crossing over were detected. The experimental data, except where otherwise indicated, were subjected to a statistical analysis according to a method of chi square described by FISHER and YATES (1949) and applying to tests of significance for 2×2 con-

tingency tables with one degree of freedom. This method is recommended where the smallest expectation of any class is less than 100. Whether the deviations from the control rate are significant at the 0.005 or 0.025 level of significance may then be determined by reference to table VIII of the above-mentioned publication. It is to be noted that for all the statistical analyses involving this method only one tail of the normal curve has been considered since deviation from the control series is in one direction only. However, if one wished to be especially conservative the levels may be doubled.

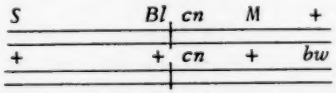
In the *Mz* series the difference in the proportion of mosaic flies as compared with the control series may not be attributed to chance as the deviations from the control rate are significant at the 0.005 level of significance.

The data provide no evidence in favor of the existence of any sex difference but, rather, a heterogeneity χ^2 test indicates that the distribution of crossovers between the two sexes is governed entirely by chance ($P > 0.05$).

Right arm crossovers have occurred in the presence of this left arm Minute. However, the magnitude is no greater than that of the control series and, therefore, no specific effect is likely. The probability that the observed deviation from an expected even distribution of crossovers between the right and left arm is due to chance is less than 0.005. The indications are, therefore, that *Mz* strongly favors crossing over in its own arm.

The data indicate, further, that a greater number of mosaics has resulted from crossing over between the kinetochore and the Bristle locus, a region 0.2 map units in length, than from crossing over between *Bl* and *M*, a region 41.9 units long. As there was no marker between *Bl* and the *M* locus it was not possible to determine how close to *Bl* the crossovers in the latter group occurred. However, these findings agree with STERN's for the third and X chromosome where it was shown that the crossovers induced by the presence of Minutes tend to occur close to the kinetochore.

MS^1 and MI^2. The following diagram schematically represents the prophase chromosomes of the experimental genotype in the case of the right arm Minutes.



Patches of white in a cinnabar eye may be expected as a result of crossing over along the right arm anywhere between the kinetochore and brown. The appearance of a *Bl*, non-*M* mosaic indicates a crossover between the kinetochore and the *M* locus. The presence of a Minute on the right arm may thus result in both eye and bristle mosaics when crossovers occur in that arm. Left arm crossovers are expressed phenotypically in non-*Bl*, *M* bristle mosaics, but their occurrence may not produce any eye mosaics. Table 2 presents the observed data.

A statistical analysis indicates that there is no evidence for the existence of a differential sex factor.

In testing the hypothesis that the controls and the experimentals are of the same population, and considering only one tail of the curve, it is found that for both MS^7 series the difference is significant at the 0.005 level ($P < 0.005$), whereas for Ml^2 the difference is significant at the 0.025 level ($0.005 < P < 0.025$).

It appears, thus, that the Minutes influence the behavior of the chromosomes in which they are located so that the frequency of somatic crossing over is increased above the control rate.

In the case of MS^7 and Ml^2 the region of crossing over could not be more precisely designated than as indicated in table 2. The presence of a white spot on a cinnabar eye indicates, merely, that crossing over had taken place somewhere between the kinetochore and the brown locus, as the phenotype of the

TABLE 2

Number and distribution of crossovers in the case of right arm Minutes. (Controls as in Table 1.)

Number of:	MS^7A			MS^7B			Ml^2		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Right arm c/o									
Between M & k	3	5	8	6	8	14	2	3	5
Between bw & k	6	5	11	4	7	11	3	3	6
Left arm c/o	1	1	2	2	0	2			0
Total c/o	10	10	21*	10	15	27**	5	6	11
		(0.34%)			(0.82%)			(0.26%)	
Total flies inspected	3076	3166	6192	1788	1486	3274	2044	2138	4182

*One double crossover scored twice; once among right arm crossovers, and again among left arm crossovers.

**Two double crossovers scored twice; once among right arm crossovers, and again among left arm crossovers.

eye mosaics produced as a result of crossing over between the kinetochore and *cn*, between *cn* and *M*, and between *M* and *bw* are indistinguishable.

On the other hand, a Minute bristle may be distinguished from a non-Minute bristle. Therefore, Bristle, non-Minute bristles may be detected, indicating that crossing over has occurred between the kinetochore and the Minute locus. Within the total of 14 eye mosaics in the MS^7 series, there were 3 which were associated with non-Minute bristles. These bristles immediately bordered the mosaic eye. Moreover, there were 5 mosaics in which only bristles were involved and these bristles were non-Minute. It was, therefore, possible to conclude that these eight mosaics developed from a genotype resulting from crossing over between the kinetochore and the Minute locus. The 11 cases of crossing over whose loci could not be more precisely defined than between the kinetochore and the brown locus were all eye mosaics.

Similar considerations have made it possible to ascribe 14 of the 27 right arm crossovers of the MS^7B series to the region between the kinetochore and the Minute locus.

In the Ml^2 series five of the eye mosaics were associated with non-Minute bristles permitting the crossover region to be limited to between the kinetochore and the Minute locus. The other six mosaics were eye mosaics only.

On the basis of what is known of the embryology of Drosophila it is possible to ascribe the presence of a combination eye color and bristle structure mosaic to a single crossover. BIRMINGHAM (1942) has shown experimentally that the eye discs give rise not only to ommatidia, but also to a predetermined area of the head hypodermis. However, it is only chance which will determine whether cells with the crossover genotype will form parts of the head as well as of eye tissue or whether they will give rise to eye tissue exclusively. The eye mosaics which are surrounded by bristles of the same phenotype as the rest of the fly offer no clue to a more precise determination of the crossover regions since Minute Bristle bristles may indicate that the crossover locus is between *M* and *bw*, or it may mean that the crossover locus is between the kinetochore and *M* but that none of the cells with the crossover genotype has taken part in the formation of the head hypodermis. On the basis of these considerations the relatively large number of mosaics indicating crossing over between the kinetochore and *M* may be taken as indication of the tendency of crossovers to occur toward the centromeric region.

The combined eye color and bristle structure mosaic is interesting for another, and perhaps more important, consideration. If, as it may be argued, the white facets of the mosaic are the result of a mutation either at the brown locus of the second chromosome or at the white locus of the first chromosome (significant in males only), then the presence of a non-Minute bristle adjoining this tissue would necessarily have to be explained by a second mutation at the Minute locus. Since the probability of such an occurrence is so extremely low it is much more reasonable to assume that crossing over has taken place between the kinetochore and the M locus. This has resulted in a rearrangement of the genetic factors to the right of the kinetochore but the *Bl* gene is still part of the resultant genotype, as it was originally.

In table 2 a double crossover has been scored for the MS^7A series, and two for the MS^7B series. Each of the three flies had a patch of tissue whose bristles were not-Minute and not-Bristle. Rather than evoke the highly improbable phenomenon of a double mutation, the situation is explained by a crossover to the left and to the right of the kinetochore. It is interesting to note that the left arm crossovers produced in the presence of the right arm Minutes were, in three of the four cases, associated with a right arm crossover. A similar phenomenon will be pointed out in the discussion on the behavior of the X chromosome.

The MS^7 and Ml^2 data were tested by means of the standard chi square test to determine whether each of these Minutes exerts a preferential effect upon the arm in which it is located. The hypothesis that the number of crossovers should be equally distributed between the two arms of the chromosome was tested and in all three instances the deviations from expectation were significant: $P < 0.01$. An arm-to-arm relationship is thus shown to exist.

The two MS^7 series will be regarded separately throughout this discussion.

Although their qualitative effects are similar, a marked influence upon the right arm of the second chromosome, the difference in the magnitude of effect between the two series is statistically significant so that the two sets of data may not be pooled ($P < 0.005$). Also, further quantitative differences will be pointed out subsequently. There is no apparent reason for the difference in the behavior of these two series of the same Minute. However, each was originally regarded as a separate entity and combined into the experimental stocks separately. Therefore, whatever differences exist in the genetic backgrounds of the two stocks may be responsible for the observed differences. This consideration leads to the conclusion that the Minute influence upon somatic crossing over is, itself, influenced by the genetic background.

Temperature experiments

In view of WHITTINGHILL's work (1937) on gonial crossing over it was decided to test the effect of heat, in the presence of Minutes, upon somatic crossing over. Both *MS*^{1}*A* and *MS*^{1}*B*, and *Mz* were used in this work. Eggs collected over 24 hours at 25°C were subjected to a temperature of 32°C for the next 48-hour period. At the end of that time, the larvae were returned to an environment of 25°C in which they completed their development. The exposure to heat was limited to a 48-hour period of the time during which the imaginal discs are rapidly growing by cell multiplication in order that the relatively brief exposure might cut down only slightly on the number of flies completing development in these cultures. A non-Minute series was carried along simultaneously. Table 3 summarizes the results obtained at 25° and at 32°C.

The heat treatment has increased the frequency of second chromosome somatic crossing over in the non-Minute as well as the three different Minute series. From a crossover frequency of 0.065% at 25° the non-Minute control frequency was increased to 0.32% at 32°. Within the *Mz* series the frequency was raised from 0.49% to 3.14%. An increase from 0.34% to 0.91% was brought about within the *MS*^{1}*A* series and the *MS*^{1}*B* series showed an increase from 0.82% to 4.64%.

For the three Minute series the differences in crossover frequency at the two temperature levels are significant at the 0.005 level of significance, whereas in the case of the non-Minute series the difference is significant at the 0.025 level of significance.

Moreover, none of the three Minute series may be considered samples of the same population as the control series at 32°, since the differences in frequency of crossing over are statistically significant at the 0.005 level of significance.

These results indicate that heat has an influence upon the processes involved in somatic crossing over and that this influence may be exerted in the absence of the Minutes.

Does the temperature shock increase the frequency of crossovers in the presence of the Minutes to the same degree as it does in their absence with the result that, in the Minute series, the number of crossovers is thereby equal

TABLE 3

Number and distribution of crossovers in Minute and non-Minute series at 25° and 32° C.

		Right arm between		Left arm between		Total crossovers	Total flies inspected
		k & M	k & bw	Bl & k	M & Bl		
Mz	25°		3	15	6	24 (0.49%)	4876
	32°		30	74	51	155 (3.14%) (76♂, 78♀)*	4935 (2119♂, 2816♀)
MS'A	25°	8	11	2		21 (0.34%)	6192
	32°	26	28	2		56 (0.91%) (20♂, 35♀)**	6136 (2780♂, 3356♀)
MS'B	25°	14	11	2		27 (0.82%)	3274
	32°	121	80	1		202 (4.64%) (91♂, 101♀)†	4355 (1956♂, 2399♀)
Control (non-M)	25°		2	0		2 (0.065%)	3061
	32°		9	1		10 (0.32%) (4♂, 6♀)	3108 (1345♂, 1763♀)

*1 fly with 2 mosaics.
**One double crossover.
†5 flies with 2 mosaics; 2 flies with 3 mosaics; 1 fly with double crossover.

to those brought about by the heat treatment plus those due to the presence of the Minute? Phrased differently, are the data compatible with an additive hypothesis or is the difference in crossover frequency at 25° and at 32° in the absence of Minutes significantly different from the difference in crossover frequency at 25° and 32° in the presence of Minutes? One might reasonably expect that some interaction has occurred between the Minute physiology and the conditions set up by the high temperature, producing some crossovers that would not have occurred in the presence of either single condition.

To answer this question, MR. H. M. HUGHES of the Department of Mathematics and the Statistical Laboratory of the University of California, Berkeley, devised a method involving the t test, in which it was assumed that the two differences are not significantly different from each other, but are considered as chance deviations in a universe of differences. The difference in percent crossing over between the rates at 25° and at 32° for the Minute and non-Minute series is determined. The difference between these two differences is then divided by the square root of the sum of the four standard errors of the difference, thus:

$$t = \frac{D}{\sqrt{\sum_{1}^{4} \frac{pq}{n}}}$$

With an infinite number of degrees of freedom the following statistical results have been determined:

$$\begin{array}{lll} Mz & t = 8.28 & P < 0.001 \\ MS^{7}A & t = 1.76 & P = 0.084 \\ MS^{7}B & t = 10.17 & P < 0.001 \end{array}$$

For *Mz* and $MS^{7}B$ the deviations based upon an additive hypothesis are significant, indicating that some sort of interaction has taken place between the conditions brought about by the heat shock treatment and the presence of the Minute, thus producing a multiplicative effect. In the case of $MS^{7}A$ the lack of significance seems to support the additive hypothesis. However, on the basis of such an hypothesis a deviation of the magnitude observed between the two differences may be expected by chance once in only twelve trials.

Reference to table 5 will show that the same patterning of crossovers along the length of the second chromosomes obtains within the 32° series as was disclosed in the 25° series. For *Mz* there is still a preferential influence upon the left arm, although a relatively large number of right arm crossovers has been produced. The probability of obtaining as great a deviation from a random distribution between the two arms is less than 0.001. Moreover, the same distribution of crossovers within the left arm is present, the greater number occurring between the Bristle locus and the kinetochore. Statistical analysis indicates that the *Mz* influence upon the second chromosome is qualitatively the same at 25° and at 32°C; the probability that they are of the same population is greater than 0.05.

In both the $MS^{7}A$ and $MS^{7}B$ series the general patterning at 32° is the same as at 25°. There was no increase in left arm crossing over, but MS^{7} seems to exert its effect almost exclusively upon the right arm despite the general increase in crossover frequency. Stated another way, the left arm remains refractory to right arm *M* influences.

One of the designated left-arm crossovers of $MS^{7}A$ occurred together with a crossover in the right arm. This double crossover was noteworthy inasmuch as each eye contained a patch of white tissue on its dorsal margin; in addition, the right and left ocellar and right and left anterior and posterior vertical bristles were non-Bristle, non-Minute. Since the probability that this mosaic has resulted from two double crossovers in one individual is extremely low, it seems more reasonable that the mosaic patch has arisen from one original cell which received the crossover chromosomes. This view suggests that the crossover in question goes back to a cell present in tissue which later gave rise to the two optic discs. Although the early embryology of the optic discs in *D. melanogaster* has not been clearly determined a statement from POULSON (1950), suggesting that a posterior thickened region of the frontal sac showing signs of bilaterality early in embryological development gives rise to the antennal-optic discs, would indicate that such an event is possible.

Again, as within the 25° series there is a marked quantitative difference between the $MS^{7}A$ and $MS^{7}B$ sets of data. However, the influence of this

Minute upon the rate of somatic crossing over and the restriction of that influence to the arm of the second chromosome in which the Minute, itself, is located is consistent between the two groups.

THE INFLUENCE OF SECOND CHROMOSOME MINUTES UPON THE BEHAVIOR OF THE X CHROMOSOME

Choice of materials

The three second chromosome Minutes discussed in the previous section were tested to determine whether the X chromosome was influenced by their presence. Flies from the cantonized Minute stocks, balanced over Curly, were crossed to Canton flies in order to derive stocks with a Minute present in one of the second chromosomes and a Canton chromosome for the other. These flies were then crossed to homozygous yellow singed^3 flies. This permitted controls and experimentals to be derived from the same cultures. The experimental flies are Minute, the control flies have normal bristles:

$$\frac{+ \quad +}{y \quad sn^3} \quad \frac{+}{M} \qquad\qquad \frac{+ \quad +}{y \quad sn^3} \quad \frac{+}{Canton}$$

Females, alone, have been inspected in this cross as the males possess only one X chromosome. Yellow is located at 0.0, singed^3 at 21.0 and the kinetochore of the X chromosome is at 66.0.

Depending upon the region of crossing over three different types of sex-linked mosaics may be expected. (Any crossing over that has occurred on the part of the second chromosome has been disregarded.) A crossover between the kinetochore and singed^3, followed by equational segregation, will result in a *y sn*^{3} spot. Crossing over between singed^3 and yellow will produce a *y* mosaic, whereas the presence of a *sn*^{3} spot indicates the occurrence of a double crossover.

Analysis of results

Controls. Among 1427 control flies there were 48 instances of somatic crossing over on the part of the X chromosome. 43 of the mosaics were *y sn*^{3} in appearance, indicating that crossing over had occurred between the kinetochore and the *sn*^{3} locus. The other 5 mosaics were *sn*^{3}. These resulted from the occurrence of double crossing over.

It is difficult to explain the absence of yellow spots, the phenotypic manifestation of crossing over between *sn*^{3} and *y*. These experiments were done very early during the course of this study and yellow mosaics may have been overlooked. It seems reasonable that they should have occurred, especially since crossovers between *y* and *sn*^{3} have taken place at least 5 times as one of the regions concerned in double crossing over. However, table 9 suggests that crossovers in this region are very rare. In this connection it is interesting to compare the results obtained by AUERBACH (1945) in studying chromosome rearrangements in somatic cells following mustard gas treatment of *D. melanogaster* eggs. Evidence from this work indicates that somatic crossing over in the distal region of the X chromosome is very rare. Also where *y* and

TABLE 4

The effect of second chromosome Minutes upon the behavior of the first chromosome.

	Total flies inspected	Number of mosaics	% crossing over
Control	1475	48	3.25
Mz	642	94	14.64
MS^{7}A	221	45	20.36
MS^{7}B	461	61	13.24
Ml^{2}	623	64	10.27

sn spots were expected in equal frequency a preponderance of *sn* spots was detected. One explanation offered for this discrepancy was that yellow spots were missed with greater ease than *sn* spots. However, since *sn* spots could have resulted from double crossovers it is possible that the total of *sn* spots was augmented by the occurrence of double crossing over. This would indicate, as does the author's work, that it is possible that double crossovers have occurred involving the region between *sn*^{3} and *y* as one of the two loci, whereas crossing over between *sn*^{3} and *y* alone, was a much less frequent event. A similar occurrence has been pointed out previously. In all cases, but one, where the left arm of the second chromosome was influenced by the presence of a right arm Minute, the exchange occurred, along with crossing over in the right arm, as one of the regions involved in a double crossover.

All mosaics were limited to the abdominal region and all were small in area, none comprising more than 5 bristles. This condition indicates that the crossovers took place rather late in development. The frequency of X-chromosomal crossing over appears to be quite high as compared with the control rate detected for the second chromosome.

Minute series. Table 4 presents the data for the control and Minute series. A definite influence by second chromosome Minutes upon the X chromosomes is indicated.

The distribution and kinds of mosaics in these four experimental series were much the same as those found in the controls. Tables 5 and 6 summarize these data.

TABLE 5

X chromosome influenced by second chromosome Minutes: Distribution of single crossovers.

	Crossover between kinetochore and *sn*			Crossover between *sn* and *y*		
	Location of mosaic					
	Abdomen	Thorax	Head	Abdomen	Thorax	Head
Mz	83	1	0	0	0	0
MS^{7}A	34	3	0	1	0	0
MS^{7}B	44	0	1	0	0	0
Ml^{2}	54	1	0	0	0	0
Control	43	0	0	0	0	0

TABLE 6

X chromosome influenced by second chromosome Minutes: Distribution of double crossovers.

Location of mosaic	Abdomen	Thorax	Head
Mz	7	3	0
MS⁷A	6	1	0
MS⁷B	15	1	0
MI²	8	1	0
Control	5	0	0

Although the three Minutes exert the same general effect upon the X chromosome in relation to somatic crossing over there are some apparent differences in the magnitude of that effect. Testing these data for homogeneity, a χ^2 value of 15.16 is obtained. This value, with three degrees of freedom, is highly significant, indicating that the four Minute series may not be regarded as samples of the same population. However, since *MS⁷A* and *MS⁷B* are significantly different from each other ($\chi^2 = 5.2$, $0.025 > P > 0.005$) it cannot be stated whether these variations are definitely attributable to the differences in the Minutes, themselves, or whether they have been brought about by differences in the general genetic background.

Nevertheless, it is interesting to note that of all four Minutes used in this study, *MI²* has had, consistently, the least effect upon the behavior of the second and the first chromosome. BREHME (1941) points out that of the Minutes that she studied *MI²* had the least effect upon growth and development; the delay in puparium formation, as compared to the wild type, being only 13 hours. *Mz*, on the other hand, produces a two days' delay in puparium formation (BRIDGES and BREHME 1944). There appears to be, then, an indication of a parallelism between the "Minute effect" and the ability of any given Minute to induce somatic crossovers.

THE INFLUENCE OF A FIRST CHROMOSOME MINUTE UPON THE BEHAVIOR OF THE FIRST CHROMOSOME

Choice of materials

M(1)o, located at 56.6, was chosen as the sex-linked Minute for this aspect of the work. *M(1)o* females were crossed to yellow singed³ males yielding the following experimental genotype:

$$\frac{+ \quad + \quad Mo}{y \quad sn^3 \quad +}$$

The control flies described in the previous section served as controls in this section of the work as well.

As previously, the crossover region may be identified by the appearance ot the resultant mosaic. A crossover between the kinetochore and the Minute locus will produce a spot in which the bristles are yellow and singed³ in appearance. This may be distinguished from a mosaic resulting from a crossover

between the Minute locus and singed[3] since, in this latter instance; the bristles will be yellow, singed[3] and Minute. Similar considerations permit the identification of the crossover regions as summarized in table 7.

Results

A total of 52 mosaics, in 381 flies examined, was produced, as compared with 48 mosaics in a total of 1475 controls. *M(1)o* significantly increases the rate of somatic crossing over of the X chromosome above the control rate, the difference in frequency being significant at the 0.005 level.

As in the control series and that in which the effect of second chromosome Minutes upon the first chromosome was tested, the greatest number of crossovers took place to the right of the singed locus. In this series, however, it was possible to narrow down the region 35.6 units, by the presence of *M(1)o*, to within 9.4 map units to the left of the kineochore. Thus, in this instance, most

TABLE 7

X chromosome influenced by sex-linked Minute: Distribution of crossovers and location of mosaics.

	Distribution of crossovers					
	Single crossover between			Double crossover between		
	kinet & M	M & *sn*	*sn* & y	kinet & M M & *sn*	kinet & M *sn* & y	M & *sn* *sn* & y
M(1)o	41	6	2	0	3	0
Control	43 between kinet and *sn*			5 between kinet and *sn*, *sn* and y		

	Location of mosaics			
	Head	Thorax	Abdomen	Legs
M(1)o	8	25	17	2
Control	0	0	48	0

of the crossing over has occurred within 9.4 map units from the kinetochore. For the other X chromosome series there is no direct evidence to indicate that the greater part of the crossovers has taken place close to the fiber attachment point. However, STERN (1936) found this to be so in his work, and since the present work indicates that most of the crossovers have taken place to the right of the rightmost marker, it is reasonable to suggest the same situation holds true here.

In the control series all the mosaics occurred on the abdomen. The crossing over induced by the second chromosome Minutes also took place, for the most part, in cells concerned with the abdominal cuticle. However, with *M(1)o* almost 50% of the mosaics were located on the thorax, and 33⅓% of the remaining mosaics were present on the head region. Similar specifically localized regional differences were described by STERN (1936). Since the imaginal hypoderm of the thorax has a different origin from that of the abdomen this difference in the distribution of mosaics may reflect a differential sensitivity

of the cells of these anlagen to the presence of sex-linked Minutes as compared with the presence of autosomal Minutes.

STERN (1936) found that the sex-linked Minutes had a greater influence upon the behavior of the X chromosome than the third chromosome Minutes he worked with. This appears not to be so in the case of the second chromosome Minutes. The percent mosaics in the presence of $M(1)o$ was 13.6%; for $M(2)S^7A$ the frequency was 20.6%. Statistically, this difference is significant at the 0.025 level of significance, indicating that the magnitude of effect of $M(2)S^7$ may be significantly greater than that of $M(1)o$.

The results of the other three autosomal Minutes were closer in magnitude to the $M(1)o$ results than were those of $M(2)S^7A$. $M(2)z$ produced 14.6%; $M(2)S^7B$ 13.2%; and $M(2)l^2$ 10.2%. It would appear, therefore, that the autosomal Minutes used here were, at least, as effective as the sex-linked Minute.

Temperature experiment

STERN and RENTSCHLER (1936) found that, as regards the X chromosome, the frequency of somatic crossing over was lower at 30°C than at 25°C. Consequently, in view of the second chromosome findings, a series of crosses was set up to test the effect of the heat shock treatment upon the X chromosome. $M(1)o$ females were crossed to $y\,sn^3$ males and the subsequent experimental treatment was the same as described for the series of second chromosome Minutes subjected to the 32° treatment. A Minute and non-Minute control series were, thus, treated simultaneously. Table 8 presents the results.

TABLE 8

A comparison of sex chromosome crossover frequency at 25° and at 32° C.

	Number of crossovers	Total flies inspected
$M(1)o$ 25°	52 (13.64%)	381
$M(1)o$ 32°	25 (4.70%)	532
non-Minute 25°	48 (3.25%)	1475
non-Minute 32°	18 (3.22%)	558

A statistical treatment discloses that the heat treatment with respect to the frequency of somatic crossing over is without significant effect upon the non-Minute flies ($P = 0.9$). Likewise, the difference in crossover frequency between the non-Minute series at 32° and the Minute series at 32° is without significance ($P = 0.28$). However, significance at the 0.005 level is attained when the Minute series at 25° is tested against the Minute series at 32°. Since the frequency is higher in the former instance, these analyses indicate that not only is the heat treatment without effect upon the X chromosome, but when heat is applied in the presence of a sex-linked Minute an interaction takes place which cuts out some crossovers that would have occurred in the presence of the Minute alone.

THE INFLUENCE OF FIRST CHROMOSOME MINUTES UPON THE BEHAVIOR OF THE SECOND CHROMOSOME

Choice of materials

M(1)o was used as the sex-linked Minute. A stock containing this Minute in the X chromosome and homozygous for *cn bw* in the second chromosome was established. Females of this stock were crossed to *S Bl cn* males to obtain the desired experimental genotype:

$$\frac{M(1)o}{+} \qquad \frac{S\ Bl\ cn\ +}{+\ +\ cn\ bw}$$

Results

Among a total of 3179 flies inspected there were 23 cases of somatic crossing over. The control frequency, as reported previously, was 2 in 3061 flies. The difference between these two frequencies is statistically significant at the 0.005 level.

All 23 crossovers involved the right arm of the second chromosome. The left arm, it seems, is refractory to most influences other than the presence of left arm Minutes.

ANALYSIS OF DATA OBTAINED FROM RECIPROCAL CROSSES

Data from the *M(2)z* crosses at 25° and *M(2)S⁷A* at 32° were analyzed to determine if differences produced as a result of setting up crosses reciprocally were statistically significant. Table 9 summarizes the results of this analysis.

TABLE 9

Data derived from reciprocal crosses.

	Total	*cn bw* × *S M Bl cn*		*S M Bl cn* × *cn bw*		Statistical data
		No. of flies	No. of crossovers	No. of flies	No. of crossovers	
Mz	4876	3777	20	1099	4	$\chi^2 = 0.20$, P = 0.65
MS⁷A	6136	4239	38	1897	18	$\chi^2 = 0.003$, P = 0.80

These data clearly indicate that the number of crossovers has been nonpreferentially distributed among the offspring of both types of crosses.

DISCUSSION

The Minutes provide rich opportunity for genetic and developmental studies. Their widespread distribution among all the four chromosomes of *Drosophila melanogaster* plus the similarity of their expression at whatever locus have made them especially interesting. The evidence presented herein indicates that the ability to induce somatic crossing over is an important aspect of the Minute

physiology. The induction of crossing over in somatic cells is not a property restricted to sex-linked Minutes or those located on the third chromosome, but three Minutes selected, with no previous bias, from the store of second chromosome Minutes have demonstrated this to be a general characteristic of the class of mutants to which they belong. Furthermore, it seems reasonable to suggest that the increased frequency of somatic crossing over depends as much upon the pairing condition of the Dipteran chromosomes as it does upon the Minute physiology. The significance of pairing during the mitotic division stages of Dipteran chromosomes is unknown as are the physiological conditions underlying the phenomenon, but it seems unlikely that crossing over between homologous chromosomes would occur under the Minute influence without the previously existing synapsis-like condition.

The arm to arm correspondence brought to light in the third chromosome study by Stern is quite clearly shown to exist by this work on the second chromosome. This ability of a genetic factor to control the behavior of the chromosome in which it is located has been met before. In maize studies of the *Ds* and *Ac* loci indicate that a heritable component may control a chromosome-break-inducing mechanism (McCLINTOCK 1951); and CROUSE (1943) discovered evidence in Sciara of an element of the X chromosome, at or near the kinetochore, which controls differential behavior.

The existence of the arm to arm relationship so precisely shown by *Mz*, MS^7 and Ml^2 gave the clue to the possible mistaken identity of the Minute in the MS^7B series. The types of mosaics in this series, interpreted on the basis of a left arm Minute, would necessarily be explained by crossing over in the left arm and right arm nonpreferentially. Since this appeared to be a marked departure from the behavior in the case of the other three Minutes, it was suggested by DR. E. NOVITSKI, that the Minute in question may not have been present on the left arm. Subsequent testing identified it as MS^7.

The work discussed in this paper points up the fact that subtle physiological differences exist within the group of the Minutes. Generally, the Minutes are able to increase the rate of somatic crossing over but certain qualitative and quantitative differences have been indicated during the course of this report.

In STERN'S (1936) analysis of somatic crossing over it was shown that the greatest frequency of crossovers occurred adjacent to the spindle fiber attachment region for both the X and the third chromosomes. The data for the X chromosome and for the *M(2)z* series are consistent with these findings. The right arm of the second chromosome was not marked to permit a more precise delimitation of the crossover region than has been indicated. However, most of the crossovers have occurred at least between the kinetochore and the Minute locus. Furthermore, it has been shown by MATHER (1939) that the heterochromatic region of the X chromosome adjacent to the kinetochore is sensitive to high temperatures, as a result of which meiotic crossing over in this region is increased. It therefore seems likely that the Minutes are able to influence the heterochromatic regions of the mitotic chromosomes in such a way that crossing over takes place at a higher rate than occurs spontaneously. Moreover, it would seem that the Minute influence is greater upon the hetero-

chromatin located on the same side of the kinetochore as the Minute itself, and only occasionally does the Minute influence override the inhibitory effect of the kinetochore upon the heterochromatin on its other side, although the influence may extend to different chromosomes. Likewise, the heterochromatin of the X seems to be more responsive to the Minute influence than is heterochromatin located in the autosomes. However, this may, in fact, represent the existence of a greater inhibitory influence upon crossing over on the part of the autosomal kinetochore. The range of effect of the second chromosome kinetochore extends to the left and to the right of the kinetochore locus whereas in the case of the X chromosome the inhibitory influence extends in one direction only since the kinetochore is located in the heterochromatic substance very close to the end of the chromosome.

In the control series at 25° and at 32° and in the series testing the X chromosome influence upon the second chromosome, all the mosaics, with one exception, resulted from right arm crossovers. Furthermore, the effect of right arm Minutes has been limited almost exclusively to the right arm, while the influence of the left arm Minute has not been so markedly limited to its respective arm. There are several possible explanations that may be offered to account for this seeming bias in the direction of the right arm and although one or two seem more plausible than others none is presented as the one correct explanation.

1. Bristle mosaics were less readily detected than eye mosaics so that the phenotypic manifestations of left arm crossing over, which gave rise to bristle mosaics only, were frequently missed.

2. The two compound eyes of Drosophila offer a greater likelihood of detecting mosaics than the area of the thorax and head owing to developmental differences that exist between them.

3. The anlagen of the cells that give rise to bristles are less responsive to the influence of agents inducing somatic crossing over than are the anlagen of the eyes.

4. In the case of the right arm, the chromosome distance within which crossing over might occur and be detected phenotypically, was greater than in the case of the left arm.

5. The situation is a reflection of a basic difference in behavior between the left and right arms of the second chromosome.

In order to examine the first possibility the number and kinds of mosaics found within the series indicated were tabulated in table 10 and the relative frequencies of eye and bristle mosaics were noted to determine whether any discrepancy exists between the number of bristle mosaics to be expected and that which was actually found.

It is not meaningful to compare the total number of eye mosaics to the total number of bristle mosaics as some types of bristle mosaics are the result of double crossing over and, as such, are expected to occur infrequently. What is more significant is a comparison of the relative frequencies of eye mosaics and bristle mosaics that are the manifestations of one particular type of crossing over. For example, in the case of the right arm Minute, crossing over between

TABLE 10

Number and types of observed mosaics.

	Eye	Eye and bristle	*Bl*, non-*M*	non-*Bl*, *M*	non-*Bl*, non-*M*	Total
Total	180	24	212	2	95	513
Mz 25°	3	0*	6	0†	15	24
Mz 32°	30	0*	51	0†	74	155
$MS^{7}A$ 25°	11	3	4	1	1*	20
$MS^{7}A$ 32°	28	8	18	1	1*	56
$MS^{7}B$ 25°	11	0	12	0	2*	25
$MS^{7}B$ 32°	80	8	113	0	1*	202
Ml^{2} 25°	6	5	0	0	0*	11
ctl 25°	2	0*	0**	0**	0	2
ctl 32°	9	0*	0**	0**	1	10

*Result of double crossover involving two arms.

**Not expected.

†Result of double crossover involving one arm.

Note: Figures in total columns are for mosaics, not crossovers and may, therefore, not agree with totals in preceding tables.

the kinetochore and *M* will result in a patch of white eye tissue if the crossover cells are concerned with formation of the eye. However, if the cells contribute to formation of the imaginal hypodermis, Bristle, non-Minute bristle mosaics arise. Table 10 indicates that from the MS^{7} series and the Ml^{2} series 136 eye mosaics and 143 bristle mosaics, of the type described, were detected. The meaning of these frequencies becomes apparent if one considers the relative likelihood of discovering eye and bristle spots. It was possible to observe a difference in eye color involving, in one case, only one ommatidium. STEINBERG (1943) has shown that each ommatidium arises from a cluster of four cells observable in optic discs as early as at 72 hours of larval life. Therefore, there were available, between the two eyes, at least 1400 such units of cells from which phenotypic manifestations of crossing over might be derived. On the other hand, for the work concerned with the second chromosome, observations were restricted to the main bristles of the head and thorax. Thus, despite the presence of large surface area capable of providing evidence of crossing over, that evidence would be detectable only if the cells specifically giving rise to bristles contained the crossover genotype. The area to which observations were restricted provided 46 bristles developing each from two cells. Thus there is a smaller number of cells capable of expressing the crossover bristle genotype than in the case of eye pigmentation. On the basis of these considerations the reported number of bristle mosaics is remarkably high and the crossover frequency induced by the presence of the left arm Minutes becomes even more significant. Is the frequency of left arm crossing over even higher than the number of mosaics indicates or does it mean that the left arm Minutes, more frequently than other conditions, influenced crossing over so early in development that the resulting mosaic areas were large enough to include one or more bristles?

Referring once more to suggestion 1, in the left arm series, *Mz*, at 25° only

3 out of 24 mosaics were eye mosaics and at 32° 30 out of 155 involved eye facets. And, in addition, in the *Mz* series at both temperatures 89 non-Bristle, non-Minute spots were found. Since this is the type of mosaic expected in the control series from crossing over in the left arm (in the region between the kinetochore and Bl), it does not seem likely that it would be missed in one case if it were detected in another. On the basis of these considerations it is not likely that the first possibility offers a reasonable explanation.

The X chromosome work indicated the possibility that a group of cells giving rise to one type of imaginal structure might have a differential sensitivity to a particular Minute influence as compared with a group of cells giving rise to another type. Thus, it may be that the forerunners of bristle cells respond differently to the presence of Minutes than do those of ommatidial cells. However, it is only in the presence of non-left arm Minutes that no or few bristle mosaics were found. In the presence of left arm Minutes crossing over in cells which give rise to bristles occurred in higher frequency than was detected for the right arm in the presence of right, left or sex-linked Minutes. Therefore, it does not appear that bristle anlagen are insensitive to Minute influences.

The control, sex-linked Minute and right arm Minute genotypes are such that crossing over between the kinetochore and the Bristle locus, a distance of 0.2 map units, is the only left arm crossover that may be detected phenotypically since Star was not usable as an indicator. On the other hand, crossing over within the entire distance between the kinetochore and *bw*, 49.5 crossover units, in the case of the right arm was phenotypically detectable. This may account, at least partly, for the absence of left arm crossovers in all series except those in which left arm Minutes were tested.

It is to be noted, however, that in the two *Mz* series 61 percent of all left arm crossovers have taken place between *Bl* and the kinetochore. Furthermore, as already pointed out, somatic crossovers tend to occur in the region of the kinetochore. Therefore, it would seem that had crossing over taken place in those series not involving left arm Minutes, an appreciable number of them would have occurred between the kinetochore and *Bl* and would, thus, have been detectable.

On the basis of these latter considerations, it is reasonable to suggest that the left arm is generally less responsive to factors inducing somatic crossing over than is the right arm. This is reflected in the appearance of only one case of spontaneous crossing over (control at 32°C) and the lower frequency of Minute-induced crossovers except when the Minute is located in the left arm itself.

SUMMARY

1. Each of three second chromosome Minutes tested was able to increase the frequency of second chromosome crossing over in somatic cells of *Drosophila melanogaster*. One Minute, $M(2)z$, is located on the left arm, the other two $M(2)S^7$ and $M(2)l^2$, are located on the right arm.

2. The right and left arms of the second chromosome were differentially

influenced. The arm in which the Minute itself was located was more strongly influenced.

3. A temperature of 32°C, applied for 48 hours to larvae which ranged in age from 0 to 24 hours beyond hatching, resulted in a significant increase, above comparable series at 25°, in second chromosome crossing over in both Minute and non-Minute control flies. The presence of the Minute and the heat treatment interacted to produce a multiplicative effect resulting in some crossovers that would not have taken place in the presence of either condition alone.

4. The three second chromosome Minutes were shown to be able to induce crossing over of the X chromosome in somatic cells.

5. *M(1)o,* a sex-linked Minute, significantly increased the rate of somatic crossing over of the X chromosome at 25°C.

6. A 32° temperature shock treatment resulted in no increase, above the 25° series, on the part of the X chromosome both for Minute and non-Minute flies. Indeed, in the presence of the Minute and the heat treatment, some crossovers were eliminated that would have occurred in the presence of the Minute alone.

7. *M(1)o,* a sex-linked Minute, at 25°, significantly increased the frequency of crossing over of the second chromosome. Crossover loci were limited entirely to the right arm.

8. Very few mosaics produced as a result of left arm crossovers were detected except where the Minute used was located within the left arm. The right arm, however, was influenced by the presence of left arm and sex-linked Minutes. Several explanations have been offered to explain the greater responsiveness of the right arm.

9. The data indicate that crossing over takes place, for the most part, at the region of the kinetochore.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1953 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

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BOSTON, MASSACHUSETTS, DECEMBER, 28-30, 1953

AGUIRRE, FRANCISCO, OUDH B. TANDON and NEVIN S. SCRIMSHAW., Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America: Distribution of blood groups in Guatemalan Indians. – The distribution of blood groups was studied with the expectation that they might be of assistance in interpreting the observed differences among racial and cultural groups in Central America. The complexity of the origin and history of the Indians of Guatemala is indicated by the 21 languages which are still in use and which comprise 7 major language groups. – The seven arbitrary regions based on the major language groups were sampled to determine the blood types of culturally and linguistically distinct Indian groups. Three to four villages were included from each region and approximately 10 individuals were studied from each village. – The differences in the frequencies of the genes studied were negligibly small among both the villages and the language groups. Consequently all data were pooled and the over-all frequencies calculated. – Among 237 cases studied the frequencies of the O, A and B genes were 0.96, 0.03 and 0.01 respectively. Out of the 86 cases studied for M and N genes the frequencies were 0.78 and 0.22 respectively. From 235 cases analysed for the C, D and E loci the following chromosome frequencies were obtained: R_1 (CDe) 0.48, R_2 (cDE) 0.28, R_Z (CDE) 0.07, R_O (cDe) 0.06, r(cde) 0.06 and R (Cde) 0.05. These results show a predominance of the O, M, R_1 and R_2 genes in the Guatemalan Indian population.

ANDERSON, EDWARD H.* and DANIEL BILLEN, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The influence of post-irradiation temperature treatment on X-ray-induced mutations in Escherichia coli. – The earlier reported observations by Stapleton and coworkers on the influence of postirradiation temperature treatment in bringing about an alteration in the survival of X-irradiated Escherichia coli has led to an investigation of the effects of similar temperature treatment on X-ray-induced mutations in several E. coli auxotrophs. – The several strains studied included purineless (P82/r), prolineless (P62), tyrosineless (T83-8), and argininеless (A45-25). To determine the number of mutations (reversion to the nonrequiring state), the exposed (40,000 roentgens) cell suspensions were plated in quadruplicate on M-10 agar plates and each set incubated at one of the following temperatures: 6°, 12°, 18°, 24°, 30°, or 37°C for 48 hours. The plates were then removed to the 37°C incubator for a period of 5 days or more. Viability determinations were made in the same

*Deceased

manner but on M-10 plates supplemented with the required nutrient. — The effects of postirradiation temperature treatment on the induced mutation rate per 10^8 viable organisms were found to vary with the strain studied. Strains P62 and P82/r both showed a maximum induced mutation rate over that temperature range in which minimal survival of the exposed cells was obtained. With strain T83-8 the induced mutation rate increased with increasing incubation temperature up to 18°C, at which point a further temperature increase had no effect. The fourth strain (A45-25) had a maximal induced mutation rate at that temperature range at which maximal survival is reached. In all strains studied, with the exception of A45-25, the "spontaneous" rate showed a different temperature response pattern than that of the induced rate. It was found that the absolute number of induced revertants was maximal in the 18°-24°C range for all four strains.

ATWOOD, K. C. and FRANK MUKAI, Oak Ridge National Laboratory, Oak Ridge, Tennessee. High spontaneous incidence of a mutant of Neurospora crassa. — In a series of spontaneous recessive lethal mutants obtained by the heterokaryon method, two occurred with high frequency. Fifteen instances of each were found among 2764 nuclei tested. The cultures from which these isolates were made had been maintained by mass transfer, and therefore the high incidence could be explained in three ways; first, by fortuitous early mutations occurring during the growth of a parent culture; second, by selection in favor of the mutant nuclei in the heterokaryon; third, by an unusually high mutation rate. Accordingly, a pedigree of cultures was started, each originating from a single conidium. This procedure immediately eliminated spontaneous mutants at random loci, but one of the high-incidence mutants has recurred 142 times in 2468 isolates from a series of 27 cultures. The pattern of recurrence is such as to suggest that a high mutation rate is an essential part of the explanation, although the role of selection has not been critically assessed. The second of the mutants which had a high incidence in the previous series has failed to appear in the present series.

BAILEY, D. W., R. B. Jackson Memorial Laboratory, Bar Harbor, Maine. The inheritance of maternal influences on the growth of the rat. — Two experiments were conducted, both using the same four inbred strains of rats. The first experiment consisted of all combinations of reciprocal crosses of the four strains; the second consisted of foster-nursing of litters in all combinations of strain of foster mother with strain of litter. Measurements consisted of gains in weight from conception to 28 days, 28 to 49 days and 49 to 70 days. Genetically determined maternal influences were found to be quite pronounced at 28 days but not so evident in the later gains. The total weights were still showing the effects of the maternal environment at 70 days. The non-genetic maternal influences were predominant at all periods of growth. The individuals' own genes had no apparent effects on the 28-day weights but became somewhat influential in the later gains. No

statistically significant interaction between the genetic maternal environments and the genetic constitutions of the growing animals could be demonstrated. A tendency was found for genetic maternal influences to be negatively correlated with genetic constitutions. The greater portion of the genetic maternal influences on 28-day weights was shown to be derived from the post- rather than the pre-natal influences.

BAKER, K. FRANCE and MYRON GORDON, Genetics Laboratory of the New York Zoological Society, at the American Museum of Natural History, New York 24, N. Y. Preliminary studies of the differential susceptibility of various strains of the platyfish to a kidney tumor. — Cystic kidney tumors, some of which show features of papillary adenoma, others of adenocarcinoma, have been found in several laboratory stocks of the Mexican platyfish, Xiphophorus maculatus. The susceptibility of the fish to these tumors appears to be genetically determined. Under identical conditions of maintenance, various pure lines derived from wild populations and from fanciers stocks have widely differing incidences of the disease. Estimates based on gross examination of hundreds of preserved specimens accumulated over several years from the laboratory raised stocks, and of currently maintained animals, indicate that the incidence of the tumors may be very high in some lines. In the Bp (Belize River) stock, derived from wild-caught fish, 18% of animals with tumors was found, in the BH "domesticated" stock 25% was found. Fishes of two inbred lines derived from the same Rio Jampa wild population are quite different in susceptibility; no tumors have been found in one of these, many in the other. Other strains exhibiting kidney tumors to a marked extent include our "Fury" and "Leopard" platyfish, and the Cp (Rio Coatzacoalcos) stock. — The exact age of tumorous animals is in most cases undetermined. No small juveniles have been seen evidencing signs of such tumors, but tumorous sexually immature fish, 7 months old, have been found. Most specimens studied have been breeding adults between 11 and 22 months old. Animals in advanced stages of tumor growth are recognized by distension of the abdomen, but normal appearing fish may also have well-developed tumors. We believe that there is an ontogenetic relationship between the growth of papillary adenomas and adeno-carcinomas in these fishes. — Attempts at transplantation of some of these tumors have been initiated, with success in some instances.

BAKER, WILLIAM K., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Evidence on the chromosome distance over which V-type position effects may act. — A cytological study has been made of certain previously reported R(+) rearrangements which cause variegation of the eye pigmentation of Drosophila virilis when they are heterozygous with the recessive mutant peach (pe), a gene located in the centromeric heterchromatin of chromosome 5. This study involved three simple 3;5 translocations each of which has the breakage point in chromosome 3 at almost the same point on the salivary chromosome, and the break in chromo-

some 5 between pe and the centromere. An examination of the relative length of the translocated chromosomes as seen in the metaphase stage of larval ganglion cells, reveals striking differences in length between comparable translocated chromosomes among the three rearrangements. These differences indicate that, although the heterochromatin may be broken at a distance away from the pe locus which is equal to at least one-third the length of the metaphase chromosome 5, the locus is still affected. The cytological results are supported by studies on the frequency of crossingover between pe and the breakage point of chromosome 5.

BERTANI, G., University of Illinois, Urbana, Ill. Prophage dosage and immunity in lysogenic bacteria. — Temperate bacteriophages (able to make bacteria lysogenic) sporadically give virulent mutants (unable to do so). Lysogenic bacteria carry phage in a non-infectious, self-reproducing form (prophage) and occasionally produce mature phage. They are immune to lysis, if superinfected with phage they liberated or other related temperate phage. Doubly lysogenic strains are obtainable, which produce two types of phage, distinguishable on the basis of one or more genetic markers. — We isolated 10 independent virulent mutants of phage P2 (J. Bact., vol. 62; Cold Spring Harbor Symp., vol. 18) of Shigella dysenteriae. These can be grouped in three classes, depending on their ability to lyse (that is, to overcome the immunity of) either only singly lysogenic cells, or both singly and doubly lysogenic cells, or neither type. It is thus possible to discriminate between singly and doubly lysogenic strains on the basis of the degree of immunity offered to a superinfecting virulent mutant, independently of the phage markers used to recognize the double lysogenic condition. This favors the hypothesis that doubly lysogenic strains carry a double dose of prophages. — More refined experiments permit discrimination between singly and doubly lysogenic strains even with weak virulent mutants (those that lyse neither lysogenic type). Weak virulent phage, superinfecting a singly lysogenic culture, reproduces inside the cells and appears as mature phage mostly between 8 and 12 cell generations after superinfection. When it superinfects a doubly lysogenic culture, its reproduction is greatly reduced and no such peak in its production is observed.

BOWDEN, WRAY M., Department of Agriculture, Ottawa, Canada. Cytotaxonomic and genetic studies in the genus Elymus, section Psammelymus Hack. — Cytological examinations of collections of Elymus mollis Trin. from many points across Canada, Iceland, Greenland, Alaska and Japan have proven that this species is tetraploid, $2_n = 28$. Numerous authors have reported an octoploid number, $2_n = 56$, for collections of E. arenarius L. from northern Europe and north-western Asia; our accessions of European origin were also octoploid. The European E. arenarius has been introduced into widely-separated localities in North America. A third species, E. giganteus Vahl, occurs in Central Asia from the Black Sea to the Amur River; both tetraploids and octoploids have been discovered. There is considerable morphological variation in E. mollis, arenarius and giganteus but the three species are distinct morphologically and geographically. Studies

are in progress with the object of detecting the numerous morphological differences between these three species and of describing the significant variations, i.e. geographical varieties, and forms. A tentative phylogenetic diagram has been prepared; the three species appear to be segregates from an ancient ancestral population which probably occurred in the Asiatic area. The Eurasiatic E. arenarius appears to be more closely related to the Asiatic E. giganteus than to the North Pacific-North American E. mollis.

BROWN, SPENCER W. and DANIEL ZOHARY, University of California, Berkeley, California. Chiasmata and crossing over in Lilium formosanum. — Inversions and large terminal deficiencies were produced, among other aberrations, by X-ray treatment of pollen. In the deficiencies considered here, either one or no chiasma was present in the deficient arm. The deficiencies showed an exact correspondence of percentage of single chiasmata in the deficient arm at diakinesis with percentage of exchange between the deficient end and the centromere at anaphase. The same relationship was observed in both heterobrachial and cephalobrachial chromosomes: ca. 70% chiasmata and exchanges for a short arm def. of chrom. A; ca. 90% chiasmata and exchanges for a long arm def. of chrom. J. (Chrom. designations from R. N. Stewart, Am. J. Bot. 34:9, 1947.) The observed frequencies do not conform to the 66.7% of chiasmata and apparent c.o. expected from a random 2-by-2 separation of the centromeres. When grown under warm field conditions, a plant of the def.-chrom.-J clone showed desynapsis, with 0-4 of the normal II's unpaired; for chrom. J, the frequencies of chiasmata at diakinesis and exchange at anaphase were both reduced to ca. 80%. With the desynaptic material, a careful search was carried out for exchange products in the unpaired, heteromorphic chrom. J's at diakinesis: although exchange products would have been highly conspicuous, not a single example was observed. (In the desynaptic material, complete pairing at pachynema and side-by-side orientation of many otherwise unpaired homologues at diakinesis indicated that a lowered frequency of chiasma formation was probably responsible for the failure in bivalent formation.) In heterozygotes of a paracentric inversion in chrom. I, the inversion II's were recognized in early diakinesis only if specific types of c.o. had previously taken place: two fragments, still closely paired, must have resulted from a 4-strand double c.o. (single fragments are not released until anaphase I); the S-configuration must have resulted from 2 external and an off center, internal c.o. The chiasma resulting from the internal c.o. is under considerable tension and can not, therefore, be explained as 2 complementary two-plane openings out within the inversion loop. Because of 1:1-correspondence of chiasmata and c.o., c.o. in the meiotic sequence must occur prior to diplonema.

BRYAN, C. R. and M. R. IRWIN, Department of Genetics, University of Wisconsin, Madison, Wisconsin. The antigenic relationships of a specific cellular antigen of Columba guinea in various species of Columbidae. — Following backcrosses to Columba livia of the hybrids and selected back-

cross hybrids from the mating of C. guinea and C. livia, a cellular antigen called C specific to guinea has been obtained in unit form. Reagents specific for the C antigen can be obtained by absorbing, with the cells of livia, antisera against the erythrocytes of backcross hybrids containing the C substance. Such reagents also agglutinated the cells of 24 other species in 12 other genera of Columbidae, indicating an antigenic similarity in these species to the C substance of guinea. The results of the agglutinations of the cells of 19 species, by the reagents produced by the respective absorptions of one antiserum to the C antigen with the cells of livia and each of these species, require for explanation the assumption of a minimum of 15 antigenic specificities (called a, b, c . . . n) of the C substance. For example, the cells of Chalcophaps indica by these tests were assigned a specificity "a" which was present in 17 of the 19 species; the combination of two specificities "ab" was found in Streptopelia humilis, "abc" in St. orientalis, "ae" in St. chinensis (Pearlneck), "ak" in St. senegalensis, and "adf" in St. risoria (Ring dove). Various tests have shown that the C antigen is antigenically related to the genetically independent substances d-4 and d-11 of Pearlneck, to the s-10 and possibly to the s-8 of Senegal. The C substance of guinea thus has many antigenic specificities, at least some of which are related to cellular antigens in other species which are genetically and serologically distinct. — These considerations lead to the question, is the C substance produced in guinea by a single gene which effects these multiple specificities, or by linked genes?

CALDECOTT, RICHARD S., Brookhaven National Laboratory, Upton, New York. The influence of oxygen tension on dormant seeds of barley irradiated with x-rays, fast neutrons and slow neutrons. — Dormant seeds of barley were placed in atmospheres of air, oxygen carbon dioxide and nitrogen for 24 hours and then irradiated with either x-rays, fast neutrons or slow neutrons. Following treatment the seeds were germinated and height measurements were taken after seven days. The atmospheres had little or no effect on the susceptibility of the seeds to slow or fast neutrons. However, the seeds treated with x-rays were strikingly affected by the atmosphere in which the irradiation was given. X-radiation either in the presence of oxygen or air reduced the seedling height by about one-half and one-third respectively over that of the seeds treated in nitrogen and carbon dioxide.

CHOUINARD, A. L., Laval University, Quebec, P. Quebec. Effects of sodium selenite on meristematic cells of onion root tip. — Onion roots were immersed for one hour in aqueous 0.05, 0.1, 0.2, 0.5 and 1.0 per cent of sodium selenite in the dark at 25°C. Fixations were made at 30 min., 1, 3, 5, and 13 hrs. Within two hours after the treatment, this compound decreases the mitotic index, the higher the concentration the lower the index. At the two highest concentrations the prophase/metaphase/anaphase ratio changes. Metaphases accumulate and irregularities appear in the spindle structure. After 30 minutes in concentrations higher than 0.1 per cent, in the cytoplasm, appear orange yellow spherical granules that give a reddish color to

the whole root. Feulgen stains these granules purplish red. (The nature of this color change needs further investigations). In non dividing and elongating cells these granules are mostly near the periphery of the cell. During mitosis, they are fewer in number and are scattered randomly in the cytoplasm except in telophase, at which time the granules accumulate at the phragmoplast and at the periphery of the cell. Possibly sodium selenite interferes with certain chemical reactions along the cell boundaries, perhaps polysaccharides metabolism.

CHOUINARD, A. L., Laval University, Quebec, P. Quebec. Heterochromatic bodies in differentiated cells of Raphanus sativus roots. — The number of heterochromatic bodies in the nuclei of differentiated cells from the fourth to the sixth mm. back from the root apex varies from 8 to 18 but is never higher nor lower than that of meristematic cells. However, there are significant differences between nuclei in the absolute and relative sizes of their heterochromatic bodies in the differentiated region, some being obviously polytene.

CHOVNICK, ARTHUR, University of Connecticut, Storrs, Connecticut. The effect of the lozenge pseudoalleles on the development of tarsal claws in Drosophila melanogaster. — A series of coisogenic stocks differing with respect to one or more of the lozenge pseudoalleles, BS, 46, and g were examined in all homozygous and heterozygous combinations. The effect of the mutant pseudoalleles on the tarsal claws was rated on a linear scale and subjected to statistical evaluation. — A position effect is seen in the examination of double and triple heterozygotes. All coupling phase heterozygotes are wild type in contrast to the repulsion phase heterozygotes, each of which exhibits a mutant phenotype to a degree determined by the particular genic combination and environmental conditions. — The effects of the pseudoalleles in homozygous condition are suggestive of physiological duplicates. — The observations on the repulsion (mutant) heterozygotes indicate that the reactions mediated by pseudoalleles are not localized at the chromosomal segment in which they are located, but result from an interchromosomal interaction. — A heterosis-like effect is seen from a comparison of the results of homozygous pseudoallelic substitution with those obtained from repulsion phase heterozygotes. — The results of this study are compared with those obtained from an immunogenetic study reported elsewhere, and the problem of position effects associated with pseudoallelism is discussed in light of the evidence presented. (This investigation was carried out at The Ohio State University, and supported by a grant from the National Institutes of Health, U. S. Public Health Service.)

CLARK, A. M., E. B. HERR, and J. V. CONWAY, University of Delaware, Newark, Delaware. Oxygen poisoning and the effects of x-radiation on Habrobracon pupae in the presence of oxygen and nitrogen. — Habrobracon when exposed to oxygen during the early pupal stages of development are deleteriously affected. A high proportion of pupae do not complete development and many of the adults that emerge exhibit wing and antennal ab-

normalities. The diploid female pupae are more sensitive to oxygen than are comparable haploid males. Habrobracon treated at larval and late pupal stages of development are not deleteriously affected. — Haploid male and diploid female pupae were x-rayed in the presence of air, oxygen or nitrogen. The deleterious effects of x-radiation were compared using adult eclosion as the criterion of viability. Comparison of the 50 per cent eclosion value for these groups shows that pupae irradiated in the presence of nitrogen are over twice as resistant as pupae treated in the presence of air or oxygen. Pupae irradiated in the presence of air are slightly more resistant than those treated in the presence of oxygen. The increase in radioresistance with nitrogen is proportional for the more radiosensitive haploid males and the less radiosensitive diploid females. — Haploid males during the pupal stage are more sensitive to x-rays, but less sensitive to oxygen poisoning than comparable females.

CURRAN, ROBERT H., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. A critical study of the cold climatic tolerance of inbred strains of mice. — With the evergrowing number of field experiments being carried on due to the development of the Atomic Bomb and Upper Atmospheric Rockets a definite need has arisen for mice which can tolerate climatic changes. With this need in mind the fifty (50) inbred strains of mice maintained here have been subjected to a cold test to determine their tolerance to cold conditions. During the winter of 1952-1953, 1400 mice were subjected to the natural cold climates found outside the laboratory. This climate ranged from -2 degrees to 35 degrees Farenheit during the months of November, December, January, February and March. Each mouse was housed individually in a 12'' x 6'' x 6'' wooden box with paper scrap bedding and regular laboratory food. In these intense cold temperatures potatoes were used rather than water bottles as a water supply. Each mouse was set out for an anticipated 30 day period and observed each day for sickness or death. Three age groups were set out in each strain, 30, 60, and 90 days, each age represented by a group of 25-50 mice. The results of this test show that each strain had a characteristic cold tolerance level which improved as the animals got older. In three races, all related, 75% of the 30 day old mice lived for the entire 30 day period in the cold, this percentage increased to 95% when the animal group was set out when 60 days of age. The remaining 47 inbred strains lasted between two and three days outside before they died. It will be noted here that two strains went into a prolonged state of coma and died within 4 hours after being set out in temperatures below 15 degrees. This experiment will be continued during the winter of 1953-54 to determine the most suitable inbred strain for experiments conducted in cold weather.

DESCHNER, ELEANOR R., Brookhaven National Laboratory, Upton, N. Y., Fordham University, New York City. The relation of break sensitivity and rejoining in Trillium following x-irradiation. — The chromosomes of Trillium erectum have been shown previously to be fifty times more sensitive to x-rays at first meiotic metaphase than at early postmeiotic

interphase. This conclusion was based on results gathered from a scoring of acentric fragments as an index of radiosensivity. From an analysis of the amount of rejoining using dicentrics and rings as an index of this phenomenon, it was shown that sensitivity was inversely associated with rejoining; that is, a higher amount of rejoining occurred in cells irradiated at early interphase than in those irradiated at first metaphase. It was decided that an investigation of breakage sensitivity and tendency of broken ends to rejoin should be carried out on other stages to establish whether this same inverse relationship between breakage and rejoining would hold true. Cells were irradiated at increasingly sensitive stages, namely, early, mid, and late prophase and the amount of breakage and rejoining scored. The amount of recombination of broken ends in the form of chromatid bridges was found to be significantly higher at later prophase. These findings seem to indicate that a high sensitivity to primary breakage is not necessarily related to a low frequency of rejoining. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

DE SERRES, FREDERICK J. and NORMAN H. GILES, Yale University, New Haven, Conn. Additional comparative studies of reverse-mutation in inositolless mutants in Neurospora. — Comparisons of spontaneous and ultraviolet induced reverse-mutation rates have been made in 7 inositolless mutants of independent origin (in addition to the 8 mutants investigated previously). Initial genetic and biochemical tests indicated that these mutants all resulted from mutation at the same locus as the 8 stocks studied earlier and are indistinguishable from each other (with the exception of one temperature-sensitive mutant). However, they have been found to differ markedly on the basis of their comparative reverse-mutation frequencies. These 7 mutants include types corresponding to at least three of the five tentative classes of isoalleles established in earlier studies. The segregation of quantitative reverse-mutability differences has been investigated utilizing the temperature sensitive mutant (83201), which is essentially stable without treatment but reverts following ultraviolet exposure, crossed with a mutant having a high spontaneous reversion rate (5202) and with one which has not reverted to date with or without treatment (WS 7-366). Comparisons of spontaneous or ultraviolet induced reversion rates in serial ascospore isolations from these two crosses demonstrate the regular segregation of mutability differences, with the same association in F_1 isolates of the temperature sensitivity and reverse mutability in characteristics as in the parental types. These results provide additional evidence for the autonomous control of mutability differences among mutants at the inositolless locus.

FORSTHOEFEL, PAULINUS F., Ohio State University, Columbus, Ohio. Developmental genetics of luxoid, a new skeletal variation in the house mouse, Mus musculus. — A study was made of the developmental genetics of luxoid, an inherited skeletal variation discovered by Dr. Margaret C. Green in the Ohio State University mouse colony. Study of the

embryology showed that the earliest obvious difference between normal and lu/lu embryos, seen in the late 11½ day stage, involved excessive growth of the anterior portions of the footplates of the forefeet and hind feet. Soon the lu/lu embryos showed extra blastemata in the footplates of their feet and deficient tibial blastemata. Histodifferentiation proceeded as in normal embryos. Origin of the kinky tail of the homozygotes was tentatively traced to vascular blebs seen in early stages which could cause deformation of somites in the tail and ultimately lead to the abnormal bony caudal vertebrae seen in the adults. Search for the possible ultimate cause of the increase in number of presacral vertebrae and the limb anomalies seen in the lu/lu animals revealed a caudad shift of the hind limb field of the order of one segmental length on the average. As a tentative unitary hypothesis, it is suggested that the primary effect of the luxoid gene is to shift the competent tissue of the forelimbs, the ribs, the sternebrae, and the hind limbs caudad. All other effects are interpreted as secondary to this primary shifting effect.

FOSTER, M. and R. COOK, Jr., Yale University, New Haven, Conn. Melanocyte population density and tyrosinase activity in mammalian skin. — Our successful attempts to demonstrate tyrosinase activity in frozen sections of normal mammalian skin lead to a tentative viewpoint which embraces not only our present positive results but also negative results reported during the past 40 years. — Preliminary observations on mouse skin suggest that tyrosinase activity, but not the dopa reaction, varies directly with melanocyte population density, dense population subsamples incubated in tyrosine showing on the average more newly formed hair bulb pigment than the components of sparse subsamples; this despite the opposite expectation if only simple competition for substrate were involved. Thus a mutual stimulatory action is indicated. — The proposed explanation on the chemical level involves the production of one or more diffusible substances capable of enhancing tyrosinase activity, quite possibly one or more intermediates in the sequence from tyrosine to melanin, i.e., dopa and/or dopa quinone. Our proposed explanation is thus consistent with the behavior of the tyrosinase system. — In short, it seems that we are dealing with a process analogous to a chain reaction on both the cellular and biochemical levels. (Work supported in part by an institutional research grant from the American Cancer Society.)

FUNG, SUI-TONG CHAN, and JOHN W. GOWEN, Iowa State College, Ames, Iowa. Sex development in Drosophila melanogaster as influenced by a gene in the third chromosome. — In 1942 a dominant third chromosome gene (Hr) causing hermaphroditism in diploid females was isolated in our laboratory. The external effects of this gene were described in earlier reports. Morphologically, the internal genitalia exhibit great variability, yet in no case is a complete sex reversal found. Each specimen represents a distinct degree of mixture of male and female parts, either rudimentary or well developed. Where genital ducts of both sexes are present, vasa efferentia as well as part of the ovarian tissue are yellow pigmented sug-

gesting that hermaphroditic gonads have the potentiality of developing yellow pigment of testicular epithelium as well as retaining the unpigmented condition of the ovary. — Transplantations of normal testes into wild type females and of female genital imaginal discs into wild type males show that only cellular contact of ovary or oviduct to testes will cause degenerative changes of the latter. It seems that some antagonistic substance is present in normal ovary or oviduct that causes degenerative reduction of male testes. The transplantations of normal male and female gonads and of genital discs into hermaphrodites and converse experiments show that development of both hosts and donors is autonomous. No sex hormones from gonads of either sex were detected. — Transplantations of normal male and female ring glands into hermaphrodites accelerate growth and differentiation of gonads and accessory organs indicating that a non-autonomous development occurs. The ring gland of normal genetic constitution must release some substance necessary for growth and differentiation which is lacking in hermaphroditic individuals.

GALINAT, W. C., University of Wisconsin, Madison, Wis. The genetics and morphology of corn grass, a macromutant of maize. — Corn grass is characterized by an active development of parts that are suppressed in the ontogeny of normal maize as well as by a restraint of other structures that usually develop. An understanding of the simple structure of the inflorescence in the original grass-like extreme of corn grass is useful as a means of interpreting the more complex maize ear. — Corn grass is highly susceptible to genetic modification as is shown by the effects of residual inheritance on the expression of the heterozygous Cg (corn grass) gene and in the modification of its segregation ratios. The modifiers have a greater effect on the expression of the heterozygous Cg gene than on its homozygote since in certain stocks this originally dominant gene segregates as a recessive. The modifiers also tend to suppress the effect of the homozygous Cg gene. These plus-modifier genes, which reduce the expression of the Cg gene, usually have some degree of dominance. One popcorn inbred, when crossed with plus-modified corn grass, has a capacity to restore the grass-like characteristics to the hybrid. In view of the primitive features of the grass-like extreme, it is suggested that the presence of a dominant minus-modifier complex in certain races of maize may be related to their phylogeny. The data will be presented in tables, by photographs and actual specimens.

GECKLER, R. P. and R. F. KIMBALL, Atomic Energy Commission and Oak Ridge National Laboratory, Oak Ridge, Tenn. The effects of hypoxia on X-ray destruction of kappa in Paramecium aurelia. — Hypoxia decreases the magnitude of effects of X rays in many organisms, including several in Paramecium. The present work demonstrates the existence of an oxygen effect on kappa, the cytoplasmically inherited material responsible for the killer character in P. aurelia. Killer animals were subjected to procedures to reduce the mean particle number to approximately one kappa particle per animal, samples of animals were irradiated under different gases and the

resulting mean particle number determined. Under a nitrogen atmosphere, at a dose of 7.7 kr of X rays the mean particle number was 0.33 per animal, while under oxygen at the same dose the mean was 0.10 particle per animal. The unirradiated control mean in air or nitrogen was 1.23. Additional experiments, in which the gases were changed rapidly before or after irradiation, showed that the critical period is during irradiation. Only under conditions of hypoxia during irradiation did less destruction of kappa occur. — Because of the interest in H_2O_2 as a possible intermediate involved in the indirect action of radiation, tests were performed on the destructive effect of this substance on kappa. No destruction could be detected in a range of concentrations from 0-40 ug/ml (5- and 10-minute exposures) although the division rate was affected to varying degrees. — These results suggest that oxygen enters into some step involved in X-ray damage to DNA-protein material in general rather than into some step peculiar to chromosome damage.

GERSHOWITZ, HENRY, California Institute of Technology, Pasadena, California. Immunogenetics of pigeons. — Two different methods were used to demonstrate that individuals of Columba livia differ in their erythrocyte antigens. The first and more conclusive method was the production of several isoantisera. The second was the development of reagents from rabbit-anti-pigeon-erythrocyte sera which detected individual differences among pigeons. Analysis of one of the rabbit reagents suggests extensive quantitative variation in the reactivity of the red blood cells of different birds. The inheritance of the antigens detected by the several typing fluids is explicable on the basis of a single gene controlling the presence of each antigen, with sub-groups controlled by alleles at certain loci, and with apparently quantitative variation of undetermined origin complicating the analysis of particular antigenic characters. (This work supported in part by a grant from the National Cancer Institute, U. S. Public Health Service.)

GERSTEL, D. U., North Carolina State College, Raleigh, N. C. Genetic segregation of allopolyploids in the genus Gossypium. — Several allopolyploids combining various species and containing contrasted genetic markers were synthesized and crossed to recessive testers. The segregation ratios reflected in part, but not entirely, known taxonomic relationships. Thus, 4n G. arboreum-herbaceum gave tetrasomic ratios for pollen color, plant anthocyanin, leaf shape and "red lethal." 4n G. anomalum-arboreum gave only rare segregants: e.g., segregation for red lethal was 282 lethals: 7 viable; for pollen color 127 yellow: 5 cream. Segregation for corolla color, anthocyanin and leaf shape genes was similarly restricted. Homogenetic pairing was therefore strong, but not complete. No segregation was obtained from G. arboreum-thurberi amphidiploids for anthocyanin, pollen color, corolla color and leaf shape factors among 81 plants (one exceptional aberrant leaf shape occurred). The comparison of two hexaploids ADD/ADD was of special interest. These came from G. hirsutum (AD/AD) and the closely related wild American species (DD) G. thurberi and G. raimondii. Segregation at the R_1 locus gave 41 red, 384 intermediate and 26 green in

the hirsutum-raimondii hexaploid, but only 8 red, 241 intermediate and 3 green from 6n hirsutum-thurberi. Similarly, segregation at the leaf shape locus was relatively frequent in the former hexaploid (27 narrow: 390 intermediate: 34 broad) but rare in the latter (6 : 110 : 3). The following are problems for further study: Comparison of ratios from reciprocal crosses and from different loci, proportion of recovered AA to aa gametes from AAaa parents, elimination at the meiotic, gametic and zygotic stages, and comparison of cytological behavior and genetical output.

GODFREY, EDWARD and H. D. GOODALE, Mount Hope Farm, Williamstown, Mass. Breeding for large size in the mouse, a progress report based on fifty-four generations of family selection. — Progress during the last ten generations has been uneven. However, evidence based upon effective selection pressures, variations in the number of matings per unit time, and changes in genetic and total variation between populations reared in a relatively constant environment suggest that continued progress is possible. — The highest average weights obtained to date were for the 51st generation, when males averaged 45.7 grams and females 37.8 grams at 60 days of age, an increase of approximately 80% over the first generation mice. Due to reduced numbers of matings fewer current offspring, and subsequent reduction of selection pressures in the next three generations, a gradual decrease to the 54th generation occurred, when the mean weights were 41.9 grams and 36.0 grams for males and females respectively. Incomplete data on the 55th generation indicates that the trend is once again upward, coincident with an increased number of matings in this generation. — From a statistical study of data on approximately 53 thousand mice, it would appear that the rate of change, as well as the magnitude of such change, depend to a great degree on the scale of breeding operations and on the persistent use of an efficient selection method.

GOLDSMITH, WILLIAM M., University of Tampa, Tampa, Florida. Ancient "Catlin Mark" Corrected with a Skull Plate. — This exhibit focuses attention to an ancient human skull (specimen #19233) in the Peabody Museum of Natural History, Yale University, bearing the legend, "Trephanned skull with silver plate from Burial Caves. Paucartamba Highlands, Peru." The exhibiter is confident from this and previous studies that the so-called "Trepannation" was congenital (either familial or individual) rather than having been chiseled out by some superstitious Witch Doctor "to let the devils out," as is claimed for such ancient practices. In order to emphasize the significance of this rare specimen, and to suggest a possible revision of the legend, various illustrations of the so called "Catlin Mark" (Bilateral Fenestral in the Parietal bones of man) are exhibited. As a rule the large parietal openings (ofttimes congenital) are separated by a median ossified isthmus as is shown in the family of the 73 year old man. However, a few cases were found, especially in younger "Catlins" where the septum was incomplete or even absent. Such seems to be the case with the Peabody skull herein considered. This congenital (not Trephanned) condition was later corrected with a metal plate—a splendid example of early skull plating.

GREEN, EARL L. and MARGARET C. GREEN, Ohio State University, Columbus, Ohio. Modification of difference in skeletal types between reciprocal hybrids by transplantation of ova in mice. — Three kinds of mice occur in the DBA and C57BL strains of mice: those with 25 presacral vertebrae, 26 psv, or asymmetrical intermediates. Ninety percent of DBA/1 mice have 25 psv; 90 percent of C57BL/10 mice have 26 psv. Reciprocal hybrids have intermediate percentages, but also are different from each other. Transplantation of the two kinds of hybrid ova into females of the BALB/c strain reduced the difference to non-significance by changing chiefly the distribution of skeletal types in offspring of DBA ova. It appears that the uterine environment influences the development of the skeleton and, specifically, that the BALB/c strain furnishes a uterine environment like that of C57BL, but difference from that of the DBA strain.

GREEN, MARGARET C., Ohio State University, Columbus 10, Ohio. A new inherited leg and foot abnormality, luxoid, in the house mouse. — A new mutation, luxoid, symbol lu, appeared in the C3H strain of mice in 1949. The gene is semi-dominant with variable penetrance in the heterozygote, the degree of penetrance depending on the genetic background. In the heterozygote the gene causes preaxial polydactyly or polyphalangy of the hind feet. In the homozygote the gene causes preaxial polydactyly or polyphalangy of both fore and hind feet, reduction in size of the tibia usually with an enlargement of the fibula, abnormalities of the tarsal bones, increase in number of ribs, sternebrae, and presacral vertebrae, and kinking of the tail. Luxoid segregates independently of agouti (a) and luxate (lx). The double heterozygote +lu+lx has normal front feet but abnormal hind limbs which may resemble those of the homozygotes lulu or lxlx or be intermediate. On a C57BL background the double heterozygotes have almost uniformly 26 presacral vertebrae, whereas lxlx mice show a high percentage with 25 and lulu a high percentage with 27 presacral vertebrae.

HARPSTEAD, D., J. G. ROSS and C. J. FRANZKE, Agronomy Department, South Dakota State College, Brookings, South Dakota. The nature of chromatin changes in colchicine-induced variants in sorghum. — After colchicine treatment of a true breeding sorghum variety, variants without changed chromosome number arose which bred true immediately. These variants were of different height, stem diameter, leaf width, seed size and yielded differently than the original untreated material. It is proposed that colchicine treatment caused the formation of a haploid chromosome complement by somatic reduction. This was later restored to the diploid number and because of its genotypical or positional advantage took over the growing point to form a homozygous shoot. Examination of meiosis in the variants, in the original material and in F_1 hybrids between these indicated no irregularity. Analysis of pairing relationships at pachytene in each of the above showed that no detectable rearrangement of the chromatin occurred. The phenotypic changes observed in these true breeding variants must then be due to minute chromatin changes of the nature of point mutations.

HARRIS, ROBERT and BERNARD PHINNEY, University of Arizona, Tucson, Arizona and University of California, Los Angeles. Auxin relations in a dwarf-1 allele of Zea mays L. — Studies were made comparing the coleoptiles of four to five day old dwarf and normal seedlings on the basis of anatomy, auxin production, auxin inactivation, and the response of decapitated coleoptiles to added auxin and to replaced coleoptile tips. — Coleoptiles of nongerminated normal and dwarf seeds are indistinguishable on the basis of length and width, and epidermal cell number. Mature dwarf coleoptiles are half the length of normals and have an epidermal cell number that is indistinguishable from normals. Elongation of both types of coleoptiles is a function of cell elongation only, at least in the epidermis. — Both auxin production and rate of auxin production are lower in the dwarf coleoptiles than in the normal. Auxin inactivation in the dwarf is no greater (and probably less) than in the normal. — Normal tips placed on decapitated normal and dwarf coleoptiles produce measurable elongation in the normal coleoptiles only. Dwarf tips placed on decapitated normal and dwarf coleoptiles cause no measurable elongation in either case. — IAA added to the tips of decapitated coleoptiles causes marked elongation of normals and no elongation of dwarfs. On a per cell basis, dwarf coleoptile sections respond half as well as do normal sections cultured in a buffered sugar solution containing IAA. — It would appear that auxin is not in itself the limiting factor in the suppressed growth of the dwarf coleoptile.

HAYASHI, J. A., W. H. STONE, K. P. LINK and M. R. IRWIN, Departments of Biochemistry and Genetics, University of Wisconsin, Madison, Wisconsin. Immunochemical studies on the J substance of cattle blood groups. — Experiments in our laboratories have established a definite serological relationship between the antigenic factor of cattle, called J, and the blood group substances related to the antigenic factors A, B and O of humans. — Studies on the J character have shown that the gene for J is expressed in the serum and may or may not be expressed on the cells. The present studies show further that water-soluble preparations from stomachs (abomasus) of cattle may exhibit serological activity. — The techniques employed were essentially the same as those applied in the isolation and purification of human blood group substances, namely, autolysis of the stomach linings, alcohol precipitation of the autolysates, followed by phenol extraction. — Preparations thus obtained from 10 individuals carrying the J^{cs} gene (character expressed in the serum and on the cells) and 2 individuals carrying the J^{s} gene (character expressed in the serum only) exhibited marked serological activity specific for the J factor and for no other known cattle blood group factors. In contrast, preparations from 5 individuals not carrying the gene for J were essentially inactive. — Preliminary chemical studies indicate that the J substance is predominantly a polysaccharide and that it is chemically related to the blood group substances of humans. — By paper chromatographic methods, the presence of glucosamine and the absence of chondrosamine were established. There were no significant differences in nitrogen content (4 to 6 per cent) between the serologically active and the inactive preparations. — Further purification and characterization of the J substance of cattle are now in progress.

HEIDENTHAL, G., Brookhaven National Laboratory, Upton, L. I., N. Y. A Comparison of X-ray Induced Dominant and Recessive Lethals in First Meiotic Metaphase Eggs and in Sperm of Habrobracon. — Methods have been developed for estimating x-ray induced dominant and recessive lethals. Natural parthenogenesis has permitted study of rates for the entire chromosomal complement. For the metaphase eggs the dominant lethal curve is a simple exponential function with the 50% lethal point at about 375r and the 90% point at about 1600r. The dominant lethal curve for the sperm is markedly different, but similar to that reported by Sonnenblick (1940) for Drosophila. The data when plotted semilogarithmically yield a curve indicating a 50% lethal point at about 1800r and 95+% beyond 4500r. Surviving F_1 virgins were tested for presence or absence of recessive lethals by means of egg hatchability counts. About 92% of all eggs laid by 192 F_1 control virgins hatched: only one female gave a reading as low as 65%. Among F_1s which had developed either from x-rayed metaphase eggs or sperm this was not the case: a proportion of these F_1s, depending upon dose with which parent had been treated, yielded counts below 60%. Such F_1s are interpreted as recessive lethal heterozygotes. The percentage of F_1s bearing one or more recessive lethals increased approximately linearly with dose from ± 6% at 500r to ± 17% at 1500r. Within this range the rates for treated metaphase eggs and sperm are about the same. F_1s from x-rayed metaphase eggs cannot be recovered for study of recessive lethals if dosage exceeds 1500r because dominant lethal rate is too high. (Parts of this work have been done at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission and also at Marine Biological Laboratory, Woods Hole, Massachusetts, Union College, Schenectady, New York. Work supported in part by A. E. C. contract No. AT (30-1) 1387.)

HERSKOWITZ, I. H., Indiana University, Bloomington, Ind. Formaldehyde-induced mutation in mature spermatozoa and early developmental stages of Drosophila melanogaster. — While mustard gas and its derivatives have proved mutagenic in both male and female Drosophila, formaldehyde mutability has been demonstrated only in males after 15-24 hours of development and in spermatozoa which were probably mature at the time of treatment. The range of stages in males mutable to formaldehyde has been extended in the present experiments. — Four different groups of individuals were subjected to 0.2% formaldehyde-treated food and male survivors tested by the Basc (so-called "Muller-5") technique for the occurrence of recessive sex-linked lethal mutations in their sperm. The ages (in hours after oviposition) at the time formaldehyde was administered and the mutation rates obtained were: 0-12 hrs., 7.7% (48 lethals in 625 tested sperm); 12-24 hrs., 4.2% (27 lethals in 644 tests); 24-36 hrs., 9.2% (27 lethals in 553 tests); 36-48 hrs., 7.6% (35 lethals in 462 tests). These data demonstrate that formaldehyde administered to males prior to 12 hrs. of development is highly mutagenic. — A modification of the vaginal douche technique, in which mature sperm are bathed with a chemical substance which is administered just after the sperm are deposited in the vagina, was employed to induce sex-linked recessive lethal mutations with a 2-10% solutions of

formaldehyde. Ten mutations in 435 tested sperm (from 6 different males), or 2.3% were obtained, proving that formaldehyde applied to fully mature spermatozoa is mutagenic. — Although suggesting no explanation for the ineffectiveness of formaldehyde in females, the results obtained show that a number of stages in males are capable of responding mutagenically to formaldehyde. (These experiments have been supported by a grant received for the work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

HERSKOWITZ, I. H., and H. J. MULLER, Indiana University, Bloomington, Ind. Evidence against the healing of X-ray breakages in chromosomes of female Drosophila melanogaster. — Rapoport (1940) reported evidence that when D. melanogaster females with attached-X chromosomes were X-rayed single breakages in heterochromatin near the centromere were capable of healing, i.e. forming new "telomeres." This type of experiment was repeated by irradiating nonbar females, having attached-X's marked by recessive genes and no Y-chromosome, with approximately 2000r and crossing them to Bar males. There were 14 nonbar male exceptions among the 17,991 sons and 129 Bar female exceptions among the 15,096 daughters examined. This completely confirmed Rapoport's experimental findings. However, breeding tests of 51 exceptional females (excluding those derived from non-disjunction in the male parents) demonstrated 6 were multi-break cases in which the distal tip of the X-chromosome provided a telomere-bearing piece for the proximal stump. This proportion is not lower than expected on the hypothesis that all the exceptional females were derived from interchanges, for the great majority would have received autosomal distal ends. As another test of whether many of the exceptions represent single breakage followed by healing, females like the above were given 1000r and 4000r and crossed to the Bar males of another, more suitable, stock. The frequencies of exceptional daughters were 0.427% (23/5387) at 1000r and 3.38% (39/1153) at 4000r. These percents, bearing approximately the ration 8:1, clearly represent an exponential increase of about the 3/2 power, as expected for interchanges. — These data constitute evidence against the occurrence in female D. melanogaster of X-ray induced chromosome breakages which are capable of healing to form new telomeres. (This work has been supported by a grant from the United States Atomic Energy Commission (Contract AT (11-1)-195).)

HOUSE, VERL I., The Johns Hopkins University, Baltimore, Md. Some observations on the interaction of comb-gap with Hairless, engrailed and alleles at the cubitus interruptus locus in Drosophila melanogaster. — Comb-gap (cg, second chromosome) produces a variable interruption of the fourth longitudinal vein and other venation effects which resemble those characteristic of the ci alleles (fourth chromosome), particularly ci and ci^W. A possible close relationship of the cg and ci loci is suggested by the following facts: there is a general similarity between ci^W and cg relative to

both the general pattern of venation effects and the response of these effects to temperature changes; cg/+ markedly enhances the L_4 interruption of $ci^W/+$, at 26° C L_4 distal to the posterior cross vein being absent in cg/+; $ci^W/+$, the pattern of venation effects in the latter genotype being similar to that characteristic of ci^W/ci^W; cg/+ and ci/+ enhance the L_4 interruption of ci/ci and cg/cg respectively; cg/+; ci/+ males show L_4 thinning or interruption at 18°C (15% penetrance), cg/+ and ci/+ being completely normal at this temperature; at 18°C cg+/+ en; ci^+/-males show 87% penetrance of L_4 interruption as compared to 21%, 0% and o% for cg+/+ en, en/+; ci^+/- and ci^+/- respectively; the combinations cg/+; ci^W/ci^W, cg/cg; $ci^W/+$, cg/cg; ci^W/ci^W and cg/cg; ci/ci are highly inviable, showing respectively 38%, 15%, 2% and 6% of normal viability at 26°C; cg, ci and ci^W show similar superadditive interaction with Hairless.

HUTT, F. B., Cornell University, Ithaca, N. Y. Sex-linked dwarfism in the fowl. — A sex-linked recessive mutation, dw, originally occurring in New Hampshires, reduces body weight of adult females by 28 to 30%. In males homozygous for dw, adult weight is reduced by about 42%. The chicks that later become dwarfs are of normal size when hatched. Growth curves show a retardation of growth by 6 weeks of age but individual dwarfs cannot be identified with certainty until 10 to 15 weeks old. The tibia and the tarso-metatarsus are disproportionately reduced in adult dwarf males. — Comparisons of dw females with full sisters of normal size show that the former begin laying at later ages, and lay somewhat fewer eggs than do their normal sisters. Viability of these dwarfs is not reduced. They reproduce normally, except for the fact that size of egg is not reduced to the same extent as size of body, so that injuries to the reproductive tract are more common than in larger birds. — Linkage tests indicate that dw is located about midway between S (silver) and K (slow feathering) in the sex chromosome.

JAMES, ALLEN P., Atomic Energy of Canada Ltd., Chalk River, Ont. Evidence of genetic segregation as a cause of sectoring in induced variant colonies of yeast. — A galactose-positive strain of Saccharomyces cerevisiae mutates from positive to negative (color change on galactose-EMB agar) with frequencies approaching 2% when irradiated with 2537 Å ultraviolet, and a high proportion of the mutant colonies are sectored for positive and negative cells. The genetic constitution of the parent line and of positive and negative portions of sectored mutant colonies has been analysed. The parent line is heterozygous since regular 2:2 segregations occur at sporulation. The positive portions of the sectored colonies appear normal, but in 6 out of 10 cases have yielded positive segregants only, indicating the acquisition of homozygosity for the dominant gene. Negative portions, when sporulated, have yielded negative segregants only. Additional abnormalities found in sectored colonies have been as follows: (1) clones from 2 negative sectors failed to sporulate but showed a mating reaction, indicating either haploidy or homozygosity of sex genes, and (2) one positive sector segregated as a polyploid heterozygous for both sex and galactose. The

data point to the occurrence of genetic segregations with high frequencies in irradiated cells (either through somatic crossing over or disjunction of homologous chromosomes instead of sister chromatids) and no plausible alternative interpretation has yet been found. As spontaneous sectored colonies have also been found, it may be that such aberrations are a naturally occurring source of genetic variation in yeast cultures.

KALTER, HAROLD and F. CLARKE FRASER, McGill University, Montreal, Quebec, Canada. The effect of parity on the teratogenic action of cortisone. – Pregnant F_1 ♀♀ from reciprocal crosses between A/Jax and C57BL/6Jax strains of mice, backcrossed to A/Jax ♂♂ and treated beginning on the 10th to 12th gestation days with 4 daily injections of 2.5 mg. of cortisone, gave birth to offspring 20.0% of which had median, post-alveolar clefts of the palate. The frequency of the defect, however, was found to vary with the parity of the ♀♀. Although the incidence gradually decreased from 31.5% in 1st litters to 5.4% in 7th-9th litters, only the figure for 1st litters is significantly different statistically from that of any other litter or for the mean (16%) of that of all other litters ($X^2 = 11.8$, d.f. = 1, $P < 0.001$). – The possibility was considered that those ♀♀ most susceptible to the teratogenic effects of cortisone also became sterile or died sooner than less susceptible ♀♀, and that their progressive elimination from the population might have caused the decreasing incidence of the defect in successive litters. No differences in incidence of the defect were noted, however, between shorter and longer lived or between more and less fertile ♀♀.

KASTEN, FREDERICK H., University of Texas, Austin. (Introduced by Theophilus S. Painter) Microspectrophotometric Determination of Desoxyribose Nucleic Acid in Adrenal Cortical Cells of Several Inbred Strains of Mice. – The adrenal cortical cells from adult females of four inbred strains of mice differing in susceptibility to mammary cancer were examined for relative amounts of desoxyribose nucleic acid (DNA). Comparative photometric measurements were made using monochromatic light (at 605 mμ) on over 200 Feulgen-stained nuclei in the zona fasciculata. Care was taken to reduce distributional errors by making measurements at approximately 50% maximum absorption and by taking measurements through the homogeneous central core of nuclei. – Determinations of relative DNA show mean values for four strains (C_3H, dba, TC_3H, C57) ranging from .843 to .922. These mean differences are statistically insignificant in four groups (dba, C_3H; dba, C57; C57, C_3H; C57, TC_3H) and significant in two other combinations (TC_3H, dba; TC_3H, C_3H). The latter show mean differences with chance probabilities of $<.00006$ and .004 respectively. Further data are being collected to verify these differences. Standard deviations range from 10 to 17.5%. Sampling errors for the data are negligible. – It is shown that strain differences in the mean DNA content of nuclei in the mature adrenal cortex are present in two out of six combinations. The relative amount of DNA in any one strain appears constant with little variation in this endocrine organ. A non-endocrine organ (kidney) in the same age group has been examined and the variation is as great (S.D. of 16.5) as was observed in the adrenal cortex measurements.

KEHR, AUGUST E. and YU CHEN TING, Louisiana State University, Baton Rouge, Louisiana. Cytological Evidence Concerning the Evolution of Ipomoea Batatas. — From our studies it has been concluded that Impomoea batatas is a hexaploid with a gametic number of X equals 45, the highest number found in the genus. During meiosis no multivalents were ever found but characteristic secondary associations involving 4-5 groups of 3 bivalents and 6-9 associations of 2 bivalents were observed. The degree of secondary association varied from intimate juxtaposition to that in which only chromatic strands connected the involved bivalents. This secondary association was interpreted to be a manifestation of chromosomes which are homologous in such minute segments that they do not form chiasmata yet have an affinity which results in their attraction to one another during Metaphase I. The authors have concluded that the sweet potato is a hexaploid of recent alloploid origin and arose by amphidiploidy of a tetraploid species (2N equals 60) and a diploid species (2N equals 30). However, secondary association suggests that total diploidization had not yet occurred.

KEHR, AUGUST E., Louisiana State University. Cycles of Transformation of Tissues of certain Genetically Controlled, Tumor Forming Nicotiana Hybrids. — The in vivo development of genetic tumors in the hybrid, Nicotiana glauca x N langsdorffii, has been well studied. Less well known are the tissue transformations of this same cross grown in vitro. This paper illustrates the in vitro changes which occur from a slow growing culture (isolated from non-tumor tissue) to rapidly growing tumor cultures. The latter may differentiate into organized tissues, and under certain conditions of a sealed environment and media manipulation can be made to revert to slow growing callus tissue. It is also demonstrated in this paper that tumor tissue may lose their ability to differentiate as well as their ability to be returned to in vivo growth and be implanted into N. glauca.

KELNER, ALBERT, Brandeis University, Waltham, Mass. Effect of reactivating light on carcinogenesis by ultraviolet light. — Since reactivating light (3600-4900 A) reduces the mutagenic and other effects of ultraviolet light (2537 A) it was of interest to see whether it would also reduce the carcinogenic action of ultraviolet light. — One group of albino mice was irradiated 5 times a week for one year with almost monochromatic ultraviolet light, 2537 A, and the other with a band of ultraviolet light 2800 to 3200 A, the most actively carcinogenic region of the spectrum. Immediately following the ultraviolet dose, half the mice in each group were further irradiated for one hour with intense reactivating light, while the other half were not treated further, and left as the dark control. After one year all treatment was stopped, but the mice were observed for four more months for the appearance of delayed tumors. — Reactivating light reduced the incidence of tumors to 60 percent of the dark control in the 2537 A group, and to 70 percent in the 2800-3200 A group. Almost the entire effect of the reactivating light was on the delayed tumors, those appearing after all treatment had stopped. The significance of these results in regard to cur-

rent theories of ultraviolet carcinogenesis will be discussed. (This work was aided in part by a grant from the National Cancer Institute.)

KEOSIAN, J., Rutgers University, Newark, N. J. and Department of Laboratories, Newark Beth Israel Hospital. Some effects on Neurospora crassa of sulfanilamide as a component of the crossing medium. — Crosses of Neurospora crassa were made in tubes of Westergaard medium containing various concentrations of sulfanilamide. Concentrations of less than 0.03% had little effect on the formation of mature perithecia. Concentrations of 0.03%, 0.05%, 0.1% and 0.2% (M/573, M/344, M/172 and M/86 respectively) increased the normal maturation time by 2-3 weeks (0.03%) and up to 9-11 months (0.1%). In 0.2% sulfanilamide, perithecia formed none of which matured, and in higher concentrations up to saturation (0.4%), few or no perithecia formed. In the sulfanilamide crosses fewer perithecia, and fewer asci per perithecium resulted, and many of the asci had one or more colorless or pale ascospores. — There was a marked reduction of the percent germination of ascospores from the sulfanilamide crosses. A large number of the ascospores from the 0.03% cross formed abnormal colonies which have persisted through many transfers. The most frequent variation was a slow, surface- or low-growing, albinistic form. Several cultures derived from the sulfanilamide crosses showed a greater resistance to the growth-retarding effect of sulfanilamide than did the parent forms. — Pretreatment of the parent strains with various concentrations of sulfanilamide before crossing in sulfanilamide-containing media gave results not differing significantly from the above. Pretreatment of the parent strains with sulfanilanide and subsequent crossing in a sulfanilamide-free medium gave results not differing from the untreated controls.

KIMBALL, E., Clinton Experimental Farm, Clinton, Conn. A multiple allelic series of primary plumage patterns in the fowl. -- Primary pattern genes are diphasic in action, and probably represent gene-clusters. Order of dominance among pigmentation phases of E, e, and e^+ are: $B > b' > b$; and among restriction phases of e, e^+, and E : $R' > R > r$. A hypothetical B-pigment series would be anticipated, viz., [B r], [B R], and [B R']. The [B r] homozygote is the well-known primary pattern (E E), extended black. Postulating rare crossovers, [B R] and [B R'] segregants were sought, but without success. It is probably that crossovers may occur 1 : 5,000, or even more rarely. Search among established breeds of domestic fowl was rewarded by demonstration of [B R] in the Birchen Game Bantam, and [B R'] in the Belgian Quail Bantam. Birchen has the phenotype, and dominant phase potentials of the (E e^+) hybrid, but breeds as a [B R] homozygote. Quail has the phenotype, and dominant phase potentials of the (E e) hybrid, but breeds as a [B R'] homozygote. The gene symbols E^R and $E^{R'}$ are proposed for birchen and quail primary patterns, respectively. The simplest and most plausible explanations for origin of E^R and $E^{R'}$ under domestication are phase-crossover or phase-mutation.

KIMBALL, R. F. and NENITA GAITHER, Oak Ridge National Laboratory, Oak Ridge, Tenn. The dominant lethal problem in Paramecium aurelia.

-- The development of genetically marked stocks has facilitated a reexamination of the failure to find dominant lethals after conjugation between unirradiated paramecia and paramecia given doses up to 10,000r of X rays. The results confirm that apparently normal conjugation occurs with some exceptions up to about this dose. Above it, various irregularities become increasingly, common. Even at high doses, there is little death in the unirradiated member, probably because, as the dose increases, failure of the migratory pronucleus from the irradiated member to function becomes increasingly common. When this pronucleus does function, then both members of the pair are usually true hybrids and fully viable. When it fails to function, the irradiated member may still be a hybrid but in this case it often grows poorly. The results are quantitatively and qualitatively the same whether conjugation occurs immediately or 10 to 20 fissions after conjugation. The data are most readily interpreted to mean that products of meiosis, containing dominant lethals are usually at a selective disadvantage, and very rarely function as migratory pronuclei. Somewhat more commonly, they function as stationary pronuclei, presumably at doses at which there are no normal competitors. All or part of the selection might be attributed to anaphase bridges interfering with movement of the nuclei. Other interpretations are possible but this one gives the most satisfactory interpretation of the data and accounts for the low frequency of detectable dominant lethals.

KOLMARK, G. and NORMAN H. GILES, Yale University, New Haven, Connecticut. Studies on chemical mutagens using the Neurospora back-mutation test. -- Comparisons of the relative mutagenic effect of a number of chemicals have been made using the Neurospora back-mutation technique. Most experiments have been performed with a purple adenineless mutant (Stanford No. 38701). Studies of the mutagenic activity of a series of epoxides indicates that diepoxybutane is the most active compound. Great differences in activity exist between certain compounds which are structurally very similar—e.g., 1,2-monoepoxybutane produces 15 times as many mutants as does 2,3-monoepoxybutane. Certain dialkylesters of sulfuric acid have also been tested. Dimethyl sulphate is highly active even in low concentrations. Genetic tests of a number of reversions induced by various chemicals indicate that such reversions have resulted from back mutation in the adenineless locus. Suppressor mutations affecting the purple adenineless locus either do not occur or are very infrequent. A double mutant, adenineless (38701), inositolless (37401) has been used for the simultaneous comparison of reversion rates at two different loci after treatment with various agents. The results indicate a marked specificity of certain chemicals for the adenineless mutant. The most striking effect has been noted with diepoxybutane. The proportion of adenine reversions to inositol reversions is 0.65 after treatment with ultraviolet light, whereas after treatment with diepoxybutane this proportion is 494.

LENG, EARL R., University of Illinois, Urbana, Ill. Expression of heterosis and apparent degree of dominance in the major components of

grain yield in maize. — Grain yield in maize has been separated into four "primary" and two "secondary" components; the expression of heterosis and apparent degree of dominance has been studied in each component and in total yield. The "primary" yield components are: number of ears per plant, kernel weight, row number, and number of kernels per row. The "secondary" components are: weight of grain per ear and number of kernels per ear. — Study of 92 different F_1 hybrids and their respective parental inbred lines showed that the hybrids were lower in ear number, nearly identical in row number, about 8% higher in kernel weight, and 42% greater in number of kernels per row than their respective "top parents." The apparent degree of heterosis was higher in the secondary components, and still higher in total yield. Also, the apparent degree of dominance was lower in the primary components than in the secondary components or in total yield. No evidence was found for the existence of overdominance effects in any of the primary components studied.

LENG, EARL R. and C. M. WOODWORTH, University of Illinois, Urbana, Ill. Effects of long-continued selection for oil and protein content in maize. — Fifty-four generations of selection for oil and protein content in maize were completed in 1953. Selection has been practiced for high oil, low oil, high protein, and low protein content. Progress in the direction of selection has continued, at varying rates, in all four selected strains up to very recent generations. The rate of progress in the two "oil" strains has differed greatly, apparently because of differences in the effects of selection on the two major components of oil content. Statistical analyses and the results of five generations of reverse selection indicate that at least three of the four selected strains still retain a considerable amount of genetic variability.

LEVINE, M., Johns Hopkins University, Baltimore 18, Md. Biochemical studies on interspecific mortality in Paramecium. — The mortality resulting from crosses between varieties (genetic species) 4 and 8 of Paramecium aurelia is due to a system of nuclear cytoplasmic incompatibility. Small amounts of the cytoplasm of each variety are antagonistic to the normal development and functioning of nuclei of the other variety, resulting in death of hybrid animals. — An enzymatic study of these varieties is in progress aimed at analyzing this incompatibility system biochemically. Some 25 different enzymes have been assayed for, some 15 have been found. These establish the occurrence of glycolysis, the Krebs cycle, and a number of miscellaneous enzyme systems in these animals. No evidence for the hexose monophosphate shunt (oxidative pathway for the utilization of glucose-6-phosphate) has been found. No enzymatic differences between these varieties are yet known. The enzymatic activities of mixtures of the breis of the two varieties are being compared to the activities of the individual breis. In the few cases thus far analyzed no inhibition or stimulation of these activities has been shown.

LOVELACE, ROBERTA, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The shattering of Tradescantia chromosomes

by ultraviolet radiation (2650 A). — Dry Pollen from Sax's clone 5 of T. paludosa was exposed to monochromatic ultraviolet radiation from a water-cooled quartz capillary mercury arc-crystal quartz monochromator setup. Precautions were taken to prevent ozone contamination from reaching the pollen. Irradiated pollen was planted on a colchicinized agar-lactose medium, fixed 20 hrs later, and metaphase stages of the pollen tube mitosis were scored for aberrations. Only chromatid and isochromatid deletions were found in tubes from pollen which had received less than 5×10^5 ergs/cm^2 of 2650 A radiation but a peculiar shattering of portions of chromosomes occurred in some of the cells following higher doses of this wave length. Often a portion of only one or two chromosomes was shattered (i.e., broken into many small fragments) whereas the rest of the chromosome set contained few or no breaks. Rarely was the whole set involved. In one experiment 1% of the cells contained shattered chromosome portions when the pollen had received 5×10^5 ergs/cm^2 while 25% were affected following 10^6 ergs/cm^2. In another experiment the following results were obtained (dosage is in ergs/cm^{2}x 10^6): 1.00, 21%; 1.76, 38%; 2.64, 57%; 4.5, 57%. Following the higher doses, more chromosomes were involved and the shattering was more extensive in the affected chromosomes. The shattering effect has not been observed following 5×10^6 ergs/cm^2 of 3130 or of 3650 A, nor after 10^6 ergs/cm^2 of 2804 A.

MARTINEZ PICO, LILIA MARGARITA, and ROBERT E. DUNCAN, Department of Botany, University of Wisconsin, Madison, Wisconsin. The effects of 5-aminouracil on chromosome structure in Vicia faba and the reversal of these effects. — Five-aminouracil produces Feulgen negative regions as a result of some interference in pyrimidine, presumably thymine, metabolism at a preanaphase event. These Feulgen negative (perhaps desoxyribonucleic acid deficient) regions, which appear as small erosions in metaphase chromosomes, become extended into long intercalary gaps traversed by strands which stain with basic fuchsin and connect the centric and proximal segments of affected chromosomes. In vicia they occur preferentially at four regions in the chromosome set. Of the two which occur in regions which can be recognized, the incidence increases in one case as the result of treatment and is unaffected in the other case. When an equimolar amount of cytidine sulfate is added to a 50 ppm solution of 5-aminouracil only 10% as many gaps are present as in the Vicia roots treated with 50 ppm 5-aminouracil alone. The anaphase groups of chromosomes are often "bell-shaped" as a result of an outward bend of the extremities of the chromosomes. The telophase and sister interphase nuclei maintain this shape. This is not as pronounced, however, in roots treated with the mixture.

MICKEY, GEORGE H., and ARMON F. YANDERS, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee and Northwestern University, Evanston, Illinois. Comparison of rates of visible mutations produced by fast neutrons and by X rays at specific loci in the third chromosome of Drosophila melanogaster. — Mutation rates were determined

for eight specific loci in the third chromosome following irradiation of adult males with either 250 kvp X rays or fast neutrons (ca. 1 Mev) from the ORNL 86-inch cyclotron. Irradiated wild-type males of the Oregon-R stock were mated to virgin females of the res stock which carried homozygously the eight recessive genetic markers, ru, h, th, st, p^p, cu, sr, and e^s. Only those male germ cells which were postmeiotic at the time of treatment were used in producing progeny. All F_1 flies exhibiting any one or more of the marker phenotypes were tested in order to distinguish between those actually involving a change at the specific locus (either point mutation or chromosome aberration) and those which were phenocopies or mimics. — Our values for both the mutation rates at the specific loci and the average rate of all eight loci resulting from X-ray treatment compare favorably with published results. Contrary to the general belief, based on studies of fast neutron effects in producing sex-linked recessive lethal mutations, that fast neutrons are less efficient than X rays, we find the mutation rate in the groups exposed to cyclotron neutrons is significantly higher per unit of dose (rep) than that in the groups exposed to X rays. This may be interpreted as reflecting a greater effect of neutrons in producing chromosome aberrations, particularly small deletions.

MITTLER, SIDNEY, Armour Research Foundation, Illinois Institute of Technology, Chicago, Illinois. Influence of Vitamins Upon Incidence of Tumors in $Tu^{50}j$ Stock of D. melanogaster. — $Tu^{50}j$ is a second chromosome recessive mutation which contributes to the production of melanotic growths in the abdomen of D. melanogaster. When reared on rigorously controlled diets, the incidence of the tumor varied with the nutrition. The flies were reared on a medium which consisted mainly of glucose, sodium ammonium phosphate, and trace elements and the yeast Hansenula anomala. Thus, practically all the nutritive factors, vitamins, amino acids, came from the yeast. Addition of large amounts of vitamins to the above vitamin-amino acid free medium altered the incidence of tumors. Riboflavin, nicotinic acid, pyridoxine hydrochloride, calcium panthothenate, ergosterol, B-12, and p-aminobenzoic acid all increase the incidence of the tumor ^{50}j. Ascorbic acid, inositol, Vitamins A and K, thiamine HCl, choline HCl, biotin, calciferol have no influence on the tumor production. (Supported by a grant-in-aid from the American Cancer Society upon the recommendation of the Committee on Growth of the National Research Council.)

MOREE, RAY, State College of Washington, Pullman, Wash. An unexpected relation between negative assortative mating and gene frequency. — Generally speaking, changes in the mating system of a mendelian population are not followed by changes of gene frequency. The exceptions are selective mating and negative assortative mating. To demonstrate the effect of the latter type of mating system, suppose a population involving one autosomal gene-pair, with complete dominance and equal gene frequencies, to be distributed according to the Hardy-Weinberg law; and suppose that -assortative mating occur on a phenotypic basis. If -assortive mating is complete, the first effect to be noted is the elimination of the homozygous

dominant genotype in the second generation, with corresponding decrease in the frequency of the dominant gene. Under other degrees of -assortative mating, the frequency of the dominant gene is reduced, but at rates not proportional to the amount of deviation from a system of random mating. The effect of different degrees of -assortative mating on the composition of populations with different initial gene frequencies, will be illustrated.

MORGAN, WALTER C., University of Tennessee, Knoxville, Tenn. Crooked. II. Increasing viability of homozygotes through hybridization. — While collecting negative linkage data from studies of seven marked chromosomes, Crooked mice were selected to start pigmented sublines. In all outcrosses of the albino inbred stock the number of phenotypically normal-tailed overlaps was increased. However, with the decrease of severity of expression in the heterozygotes there was a corresponding decrease in severity of secondary lesions in the Cd homozygotes. A high percentage of the pigmented homozygotes possessed incisors. With this improved condition the homozygotes grew on a scale comparable to that of their normal siblings. This proved that extreme growth retardation of the inbred SMALLs was probably attributable to non-eruption of the lower incisors and subsequent malnutrition. — As in the albino SMALLs, numerous eye lesions were observed. A criterion for identifying Cd/Cd mice at birth has been discovered. All mice with pigment-reduction in the iris have proven to be homozygotes. Although the degree of pigment-reduction ranges from 0 to 100, a correlation has been established between amount of reduction and degree of microphthalmia in adults. — Inasmuch as some homozygotes have no pigment-reduction of the iris, the most valid criterion for identifying all of these mice is by observing the tail-fur of the 14-day mouse. The same anomalous tail-fur and abnormal pelage reported for the albino stock has been observed in pigmented homozygotes. — For the first time Cd/Cd females have produced offspring. Thus, data have been collected from matings of Cd/Cd x Cd/Cd which further verify the hypothesis that the albino SMALL mice were the homozygotes. (Research conducted at Nevis Biological Station, Irvington-on-Hudson, New York.)

MOSES, M. J. and J. H. TAYLOR, Brookhaven National Laboratory, Upton, N. Y. and Columbia University, New York, N. Y. — Desoxypentose nucleic acid synthesis during microsporogenesis in Tradescantia. — The simultaneous estimation of desoxypentose nucleic acid (DNA) content of single cells and the localization of P^{32} incorporation by autoradiography provides a unique opportunity to study the dynamic aspects of synthesis in two parts of the DNA molecule during cell division. This paper reports the correlation between time of P^{32} incorporation in the DNA fraction and time of increase in amount of DNA per cell during the meiotic and two mitotic divisions of microsporogenesis in Tradescantia. Healthy inflorescences were allowed to take up to P^{32} from a nutrient solution and after 20 hours buds were fixed and sectioned. Alternate groups of sections were prepared for autoradiography (Taylor, J. H.; Exp. Cell Res., 4:164-172, 1953) and for cytophotometric estimation of the DNA (Feulgen) content of

nuclei. In general a constant relationship was observed between the amount of DNA per cell and chromosome number. However, prior to any of the divisions, DNA amounts had doubled before evidence of chromosome doubling was visible. Three brief periods occurred when increasing DNA values were found: early meiotic prophase (leptotene), late microspore interphase and early pollen interphase in the generative nucleus. It is assumed that these represent periods of pre-division DNA synthesis. P^{32} was found to be incorporated in the DNA fraction only at those stages where the increasing DNA values were observed. It thus appears that DNA synthesis, involving at least the sugar and phosphorus portions of the molecule, occurs relatively rapidly and at specific times during the cell cycle. Timing of the synthetic period at interphase is not necessarily related to cytological criteria of division but appears to vary with the cell type. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

NAYLOR, JAMES M., (introduced by G. Sander), University of Wisconsin, Madison, Wis. Cytological studies of the effects of plant growth substances in lateral bud inhibition. — Lateral buds of Tradescantia paludosa were studied cytologically to determine whether the inhibition of the bud meristem through natural auxins or synthetic plant growth substances involves a control of desoxyribose nucleic acid synthesis as well as mitosis. In axillary buds inhibited by the terminal bud, mitoses are completely absent in a group of cells constituting initials of both tunica and corpus at the distal portion of the apex. Photometric measurements indicate that the DNA (Feulgen) content of interphase nuclei in this "zone of inhibition" is uniformly held at the amount characteristic of the diploid telophase or early interphase (2 C), despite differences in nuclear size. In the subapical tissue of axillary buds, mitoses occur with a low frequency. Upon removal of the vegetive shoot apex this mitotic rate increases. At the apex of the released bud a doubling of the DNA content occurs in the interphase nuclei of the previous "zone of inhibition," followed by mitosis. This suggests that apical dominance involves auxin control of interphase processes leading to DNA synthesis in axillary buds. Naphthaleneacetic acid, applied to the cut surface of decapitated plants, may substitute for natural auxins in the inhibition of both mitosis and DNA synthesis. (Work supported by grants to Professor C. L. Huskins from the American Cancer Society, upon recommendation of the Committee on Growth, N.R.C., from the Research Committee of the Graduate School (W.A.R.F.), and from the Rockefeller Foundation.)

NEWCOMER, EARL H., University of Connecticut, Storrs, Conn. Chromosome number, morphology and the cytology of sex of the domestic fowl. — The chromosome number of the domestic fowl has been variously reported as having a somatic number as low as twelve to a high of eighty. Since genetic evidence supports an X-O type of sex inheritance with the female being heterogametic, it is presumed that the female possesses one less chromosome than the male, and this unpaired chromosome has been

associated with virtually all of the larger chromosomes by the various investigators at different times. — By means of a smear technique with a new fixing fluid, it is shown in this study that the chromosome complement of the domestic fowl consists of six pairs of large or macro-chromosomes, which presumably correspond to the six known linkage groups, and a variable number of small or micro-chromosomes which we have classed as super-numeraries. These latter are heterochromatic, aberrant in behavior, and apparently possess not a localized but a diffuse kinetochore. The sex chromosome could not be identified but the evidence suggests that none of the six macro-chromosomes are unpaired in the female.

OGUR, MAURICE, GERTRUDE LINDEGREN, and CARL C. LINDEGREN, Southern Illinois University, Carbondale, Ill. Respiration Deficient Yeasts. — Inability to utilize lactate is associated with respiration deficiency based upon enzymatic deficiencies of flavoprotein and cytochrome. Respiration negative diploids, by mating respiration negative by respiration positive haploids, yield four respiration negative clones with other markers segregating 2:2.

PARTANEN, C. R., Harvard University, Cambridge, Mass. Nuclear changes in unorganized plant growth in vitro. — The nuclear condition in unorganized plant tissue cultures has been compared with that in the organized plant grown in vitro. The studies were carried out primarily with haploid fern gametophytes, their various expressions under varied cultural conditions, and with certain spontaneously occurring tumor-like tissue cultures that have been isolated from the gametophyte cultures. — The studies indicate that in the temporary, i.e., reversible, abnormal expressions the nucleus remains apparently normal with respect to chromosome number and mitotic behavior despite prolonged abnormal growth. However, in those forms which show a gradual loss or modification of an initial limited ability to produce organized growth, there is also a gradual nuclear change from an apparently normal condition, with the haploid number of chromosomes, to a variable aneuploidy in the triploid to the tetraploid range, which is further characterized by hypercontracted, disoriented chromosomes at metaphase. The increase in chromosome number appears to occur by means of a process resembling endomitosis.

PATAU, KLAUS, University of Wisconsin, Madison, Wis. DNA Constancy. — The hypothesis of a constant DNA content per genome implies that continuous DNA variation occurs only as part of a precise DNA doubling in the course of a complete genome reproduction. Anueploidy and other irregularities will cause odd DNA contents. Statistical analysis of replicated DNA determinations (Feulgen) by the photometric two wave length method permits sensitive tests of the hypothesis. In measurements on human spermatozoa systematic errors can be virtually eliminated. There is no indication of any DNA variation between nuclei with equal chromosome complements. If such variation should exist, the coefficient of variation would be significantly less than 1.8%. In technically less favourable material the measured DNA contents of com-

parable nuclei display some variation beyond the photometric reading errors. It has coefficients of variation of less than 5% both in mature salivary gland nuclei (which have undergone about ten DNA doublings) and prophase nuclei of onion. It can plausibly be ascribed to variation in nonspecific absorption. No significant DNA changes are found during mitosis in onion (mean DNA value of 15 metaphases: 98.9%; of 11 anaphases: 105.2% of that of 19 prophases). It seems unlikely that the cell could regulate its DNA content that accurately if DNA were not organized as a self-reproducing constant structure which may be the gene or a template upon which the physiologically active gene is formed. Thus accurate DNA measurements suggest similar conclusions as genetic DNA effects in pneumococci and radioisotope work on phages.

PEARSON, HELEN M. and O. H. PEARSON. Eastern States Farmers' Exchange, West Springfield, Massachusetts. Dimorphism in Cucurbita moschata. — The Butternut variety is reportedly a variation of Canada Winter Crookneck, appearing about twenty years ago. Reversion to the Crookneck variety has been frequently observed. In 1948, dimorphic plants were found, the basal branches bearing short-necked fruit (Butternut), the main branch and later developing laterals bearing long-necked fruit (Crookneck). In 1950, on one such plant, two self-pollinated fruits were secured, one of each type. Their progeny showed all three types, short, long and dimorphic, but the progeny from the long type were predominately crookneck or dimorphic. Tip cuttings from a Butternut type branch of a dimorphic plant, and from a crookneck type branch from another dimorphic plant have been carried from 1951 to date, with no further variation. Four tip cuttings from each of three dimorphic plants were made in August 1952. During the winter all four cuttings of two of the clones reverted, although the fruits borne on the lower branches of the original plants after the cuttings were taken from them were of the Butternut type. During 1953 these have remained constant for the long type. Three of the four cuttings of the other clone reverted, but the fourth and most basal has remained Butternut in type. — Long x Long reverted types show a low percentage of Butternut in the F_1. Approach grafts made between the horticultural varieties Butternut (scion) on Crookneck (stock) showed no effect in three of four cases. In the fourth graft dimorphism developed in the scion, although it did not appear in any other part of the scion-plant. — These phenomena appear to be the result of an unstable genetic complex, and further studies are in progress.

PERRY, T. O., University of Florida, Gainesville, Florida. The Genetics of the Photoperiodic Response in Poplar Tree Species. — When grown under uniform environmental conditions ecotype collections of poplar species show great diversity in date of cessation of terminal growth. This diversity is correlated with the various latitudes and frost-free seasons within the range of the species. Hybridization between ecotypes and artificial alteration of the photoperiod shows that the duration of terminal growth is controlled by the interaction of the genotype and the

photoperiod. — In response to the selection pressure of seasonal variation in temperature, poplar tree species have evolved a genetic system to control their duration of seasonal growth and initiation of frost resistance. Photoperiod, the only factor of the environment with a uniform seasonal variation which is constant from year to year, is the factor which evolutionary pressures have selected as the dominant agent for controlling duration of growth and onset of frost resistance. — The determination of growth duration and frost resistance by heredity and photoperiod places severe restrictions on the possibility of successful relocation or transplanting of tree seed. In some cases transfers can be made over distances of hundreds of miles and in others as little as 20 miles will result in decreased growth. In the northern hemisphere plants may be moved northward if the growing season in the new environment is long enough; but they cannot be moved southward without loss of growth, even if the growing season is longer than in the northern environment. Similar restrictions are encountered in moving plants to localities of similar photoperiod but different frost free seasons.

PETERSON, PETER A., University of California, Citrus Experiment Station, Riverside, California. A Mutable Pale Green Locus in Maize. — A study was made of the mutable pg gene in order to analyze the mechanism underlying its instability. This mutable locus was found in testing the effects on maize kernels of irradiation from the Bikini atom bomb. Two distinct seedling phenotypes are associated with the pg complex; a stable-type (pg^s) characterized by its uniform "pale green" color with occasional sectors of green stripes and a variegated-type (pg^m) containing numerous dark green stripes on a pale green background. These green stripes were found to represent mutations of pg to the normal Pg allele. These two pale green phenotypes appear, together with the normal green plants, in three characteristic ratios in the F_2 progenies: (1) 3 : 1 for green to stable pg- the s class; (2) 12 : 3 : 1 for green to mutable pg to stable pg—the m & s class; and (3) 3 : 1 for green to mutable with a low and variable fraction of stables—the m class. There are various lines of evidence to show that the m & s class is due to the presence of an independently segregating factor, En (Enhancer), which increases mutability at the pg locus. The mutability of the m class is autonomously controlled—i.e. En is located adjacent to the pg locus (pgEn). In addition to the m-type F_2 progenies obtained in outcrosses with pgEn, two unexpected classes—the m & s and s—also appear. Their frequency is interpreted as indicative of a high rate of change from pgEn (m class) to pg En (m & s class) and pg (s class). Transposition of En from its position adjacent to the pg locus results in its appearance at another position in the chromosome complement. In addition to the location of En adjacent to the locus and on an independently segregating chromosome, En was found in one instance to be linked with pg at a distance of approximately 36 cross-over units. This third location of En is further evidence that it undergoes transposition. pg^s mutates to pg^m in a low frequency (1/420). pg^m mutates to pg^s at the rate of approximately 2.5%—4% in the gametes of pgEn P_1 pollen parents. — The mutable locus

is hypothetically interpreted to represent the association of an inhibitor (I) with the normal dominant allele Pg resulting in a pale green phenotype. The loss of (I) under the influence of En is manifested in the mutation of pg to Pg.

PITTENGER, THAD H., California Institute of Technology, Pasadena, California. Pseudo-wild type strains in Neurospora. – Phenotypically wild-type progeny from crosses between closely linked genes in Neurospora crassa were genetically analyzed to determine whether pseudo-wild type (PWT) strains were present similar to those previously described involving mutants in the D linkage group. 654 phenotypically wild-type progeny were analyzed from 17 crosses and 99 different PWT's were found involving mutants in each of the five linkage groups tested. – These PWT's have the following characteristics. They are phenotypically wild and are derived from normal-sized ascospores from the progeny of crosses between closely linked genes. In crosses of PWT's with standard wild types approximately 50% of the outcross progeny are mutants. It can be shown that both of the original parental mutants, if they are distinguishable, are recovered in the outcross progeny unless one of the parental mutant strains carries a linked translocation in which case only one of the parental mutants is recovered. Mutant and wild type macroconidia were recovered from all PWT's tested. All PWT's were "unisexual." Although PWT's are heterocaryotic for two linked mutants, no PWT's have ever been found in which a marker gene in an independent linkage group was heterocaryotic. – The recovery of 99 different PWT's from five different linkage groups indicates that the phenomenon giving rise to such strains is one of widespread occurrence and should be given careful consideration in evaluating genetic data involving closely linked or possibly allelic genes. The phenotype and behavior of these PWT's is consistent with the suggested explanation of their origin from unstable disomic nuclei.

PITTMAN, D. D. and CARL C. LINDEGREN. Southern Illinois University, Carbondale, Ill. Population dynamics in the long-term adaptation of Saccharomyces chevalieri to galactose. – Saccharomyces chevalieri ferments galactose by "long-term" (six day) adaptation. "Short-term" adaptive fermentation requires less than two days; mutation followed by selection causes fermentation in from six to twenty days. About seven per cent of the cells of S. chevalieri are respiratory-deficient "petite" (lactate, pyruvate, acetate, formate negative). Both normal and "petite" colonies contain galactose fermenter papillae on a background of galactose-negative cells on galactose agar. Papillae on normal (lactate, pyruvate, acetate, formate positive) colonies soon become confluent. Nearly one-third of the "petite" colonies contain no papillae; papillae on "petites" do not deadapt to "long-term" fermentation. They are stable, "short-term," respiratory deficient mutants. Mutation rates calculated by the Poisson method showed the change from the inability to ferment galactose to be 3×10^{-7} mut/cell/gen. on galactose agar. Variance analyses showed the rate of change as 0.4×10^{-7} in glucose. Experiments indicate that mutation and cell division

are separable phenomena. — Similar "short-term" respiratory mutants occur and the gene controlling galactose fermentation segregates at meiosis. In mixed populations respiratory non fermenters outgrew the other three types in glucose. Long-term adaptation to galactose by S. chevalieri is due to mutation and selection; reversion to "long-term" adaptation is due to the selective advantage of the respiratory non fermenter in glucose.

PREER, J. R. and R. W. SIEGEL, University of Pennsylvania, Philadelphia. Cytological demonstration of paramecin in Paramecium aurelia. — "Paramecin," a toxin produced by "killer" paramecia, causes death to "sensitive" paramecia. Killers possess several hundred genetic cytoplasmic particles called "kappa" which are necessary for the production of paramecin. A small proportion of the cytoplasmic bodies which have been identified with kappa contain one or more refractile regions. Bodies with these regions are called brights. We have found that only those strains of paramecia which produce the toxin paramecin contain brights. Although animals of other strains unable to produce paramecin may contain kappa-like cytoplasmic bodies, they lack brights. The sedimentation rates of paramecin and brights are indistinguishable, while the sedimentation rate of non-bright kappa is different. It is concluded that brights are the bearers of paramecin activity and that they are produced by non-bright kappas.

RAY, DAVID T., Howard University, Washington, D. C. Production of eye-color mutations in Mormoniella by low x-ray doses and a dose-action curve. — Unmated females x-rayed with doses ranging from 1340 to 5360 r have given bright eye-color mutant sons in percentages ranging from 0.14 to 0.85. Similar mutants are found less frequently at lower doses. Data are thus far consistent with direct proportionality.

REDFIELD, HELEN, Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia 11, Pa. Crossing over in the left end of the X chromosome of Drosophila melanogaster and its relation to cytological length. — The free end of the X shows a marked disproportion between distances measured by simple crossing over and the distances between the same genes as measured by counting bands in salivary preparations; thus there is here great variation in Bridges' "coefficient of crossing over." Comparisons were made of the crossing over in the yellow-white region (1.5 crossover units, 94 ± bands) with that of the adjoining white-split region (1.5 units, 5± bands) in eight types of female in which the crossover relations were altered by combinations of the heterologous autosomal inversions associated with the "Curly" (II) and the "Payne" (III) chromosomes. The increases produced in the left end of the X vary from 75% of the control value for the Curly subcultures to approximately 500% for the Curly Payne first cultures; in each case with minor variations the increase in the yellow-white region is similar in magnitude to that in the white-split region. The higher crossing over in the presence of heterologous inversions thus depends upon a mechanism distinct from that responsible for the discrepancies between genetic length and physical length in these regions.

REISNER, ALEX, R. W. BARRATT, and DOROTHY NEWMEYER, Stanford University, Calif. Confirmation of the seventh linkage group of Neurospora crassa. — M. R. Emerson and S. Emerson (unpublished) showed that the sulfonamide locus (sfo) is not located in linkage groups I through VI and therefore designated sfo as the first locus in the seventh linkage group. Recently two other loci, thiamin (thi-3) and nicotinic-tryptophan (nt) have been found to be linked to sfo and unlinked with markers on the other six linkage groups. thi-3 (isolation number 18558) gave no recombination with sfo in 46 asci and 6% recombination (calculated from wild type frequency) in 201 random isolates. nt (isolation numbers 65001, 39401, C86) gave 27.3% recombination with sfo in 33 asci (15 parental ditype, 18 tetratype). nt and thi-3 gave 27.7% recombination in 71 asci (33 parental ditype, 37 tetratype, 1 non-parental ditype) and 20.2% recombination in 243 random isolates. — In these crosses the centromere distances of thi-3 and nt were respectively 1.2 units (83 asci) and 20.0 units (110 asci, including 40 unpublished obtained from L. Garnjobst). Added to the data summarized by Barratt, Newmeyer, Perkins, and Garnjobst (Advances in Genet. 6) this gives centromere distances of 3.4 (148 asci) and 22.1 (238 asci) respectively. — Statistical tests on crosses involving markers close to centromere show that thi-3 is independent of each of the other six linkage groups ($P = < 0.01$). Crosses of nt to markers in the other six groups also indicate independence ($P = < 0.05$), but do not exclude location in possible left arms of groups II through VI because of the large centromere distance of nt. — Scoring of sfo is somewhat laborious, and also suppressor mutations occur frequently; therefore thi-3 (readily scorable on minimal agar slants) and nt offer more satisfactory markers for testing linkage to group VII.

RIZKE, M. T. M., Dartmouth College, Hanover, N. H. A histochemical study of hereditary melanosis in Drosophila willistoni. — The lethal homozygotes of 42P develop pigmented spots in the cuticle. These spots are small, dark brown or black hardened regions or sclerites which appear after the larvae have outlived the normal larval period. The expressivity of the pattern is 30% when the larvae are raised on cream of wheat-molasses medium with yeast. In addition to this abnormality the lethal homozygotes can be identified by their aborted imaginal disks, absence of haemocytes and undeveloped ring gland. The male gonads continue to develop and contain spermatids and spermatozoa but the size and stage of development of the ovaries are comparable to the ovaries of normal larvae. Often the 42P larvae develop internal melanosis of the gut and pericardial cells in addition to the integumental pigmentation. — The genesis of the 42P spots was compared with normal cuticle using the Millon reaction, the periodic acid-Schiff reagent with acetylation techniques, the chitosan test, and staining with Mallory triple stain, fast green, Azure B and Giemsa. — Normal larvae and larvae of 42P were treated with dopa, tyrosine and phenylalanine. The integument of 42P larvae which were not yet visible spotted developed pigmented spots when placed in dopa solution in addition to blackening of the torn edges of the larvae. No spots appeared in normal larvae; they became blackened at the torn edges and showed a slight diffuse dark-

ening of the cuticle. The integument with the induced melanotic spots of 42P was fixed, sectioned and stained. These spots are similar in appearance to the spontaneous 42P spots. This study indicates that an upset of the mechanism of cuticular darkening and hardening is associated with the 42P factor.

ROTHENBUHLER, WALTER C., JOHN W. GOWEN, and O. W. PARK, Iowa State College, Ames, Iowa. Action of Eye-Color Mutations as Revealed in Mosaic Honey Bees (Apis mellifera L.) — Four recessive mutations at separate and non-linked loci were studied in their mosaic combinations with wild-type (black) tissue. They were ivory (i), cream (cr), and snow (s), all producing white eyes, and chartreuse (ch) producing yellow-green eyes in young bees. Eye-facet size demarcated wild-type tissue which was female, diploid, and heterozygous for the mutant gene, from genotypically mutant tissue which was male, haploid, and hemizygous for the mutant gene. Mosaics of wild type with ivory and mosaics of wild type with snow are visually wild type in pigmentation at the edge of the ivory or snow tissue adjoining the wild-type area. Mosaics of wild type with cream and wild type with chartreuse show no such border effects. The development of pigment in the border facets of ivory and snow tissues adjoining wild type reflects the competence of these mutant tissues to react with diffusible substances produced in the wild-type tissue. The lack of border pigmentation in cream and chartreuse areas reflects the incompetence of these tissues to react with wild-type diffusible substances. — Ivory female tissue mosaic with chartreuse male tissue apparently leads to wild-type pigmentation in the edge of the ivory tissue, but to no change in the chartreuse area. Other mosaic combinations are under study. — Studies of mosaic individuals show that the honey-bee mutant genes ivory and snow are non-autonomous, whereas cream and chartreuse are autonomous in their effects.

RUDKIN, G. T., HOWARD TEMIN, and JACK SCHULTZ, Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Penna. Evidence for the equality of chromosomal material in white and wild-type bearing sections of salivary gland chromosomes of D. melanogaster. — A study of the cytochemical characteristics of genetic change at the white locus has been made by ultra-violet microspectrophotometry. Section 3 and subsections 3A, C and D were compared in salivary gland nuclei of female larvae heterozygous for the wild-type allel of Oregon R, and a white (w^{48h}) derived from it, carried in cytologically distinguishable chromosomes. Total extinction at 275 mμ and 257 mμ was measured by modification of the photographic method of Caspersson. The chromosomes fell into two groups, the mean of the total extinctions for the one group being roughly twice that for the other. Within each group, the variance of extinctions at 275 mμ was greater than the variance of the extinctions at 275 mμ. Since the two extinctions are functions of the relative amounts of nucleic acid and protein present in the chromosome, the protein moiety (extinction peak near 275 mμ) is considered to be more variable than the nucleic acid moiety (extinction peak near 257 mμ), a result either of a

variable fixation process (50% acetic acid) or of a variable physiological state of the chromosomes, or both. Nevertheless, the subsection containing the white locus (3C) was statistically identical in white and wild-type bearing homologs at both wave-lengths. The difference between the means for w^w and w^+ is ± 2% at 275 mμ, ± 1% at 275 mμ, setting the upper limit to the difference between the homologs at less than one tenth of a band similar to 3C3. The cytological difference between the white mutant and wild-type, confirmed in this material, is thus not accompanied by a quantitative difference in ultra-violet absorbing material.

RUSSELL, ELIZABETH S., KURT I. ALTMAN, KURT SALOMON, and JAMES K. SCOTT, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and Depts. of Radiation Biology, Biochemistry, and Pharmacology of the University of Rochester, Rochester, New York. Chemical Characterization of the Erythropoietic Defect in W^vW^v Anemic Mice. — Hemoglobin synthesis in normal ww, slightly anemic W^vw, and severely anemic W^vW^v was compared, using α-C^{14} glycine. Two to four month old littermates were injected intraperitoneally with 2μc. α-C^{14} glycine per 100 gm. body weight, sacrificed after 2-33 days, and in each group blood from 3-4 animals of identical genotype was pooled. Great differences were found in C^{14} activity of protoporphyrin dimethyl ester isolated from hemoglobins of the three genotypes. In ww, C^{14} activity was present at 2 days, very high at 3, and incorporation was maximum at 7-33 days. In W^vW^v, no C^{14} activity was present at 2, 3, or 7 days, but appreciable amounts were found at 22 and 33 days. W^vw gave an intermediate picture. No comparable differences were found in rate of globin synthesis. The life-span of erythrocytes from all three genotypes appears identical. The great delay in protoporphyrin C^{14} incorporation and the storing of C^{14}-containing intermediate (s) later available for protoporphyrin synthesis make it probable that the anemia of W^vW^v, known to involve a maturation arrest, either (1) arises as a consequence of the protoporphyrin synthesis defect, or (2), more probably, that the arrest of erythropoietic maturation occurs at a stage critical for protoporphyrin synthesis.

RUSSELL, LIANE BRAUCH and MARY H. MAJOR, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Dominant lethals in mouse oocytes induced by X rays in air and in 5% oxygen. — Mature (101 x C3H)F_1 females, after 10 minutes in a lucite chamber flushed with air or (5% O_2 + 95% He), were irradiated with 400 r X rays in the same gas or left an equivalent length of time without irradiation. The body anterior to the ovaries was shielded. Females were placed with males immediately after exposure. All copulated within 5 days, and there was no significant difference between controls and experimentals in the proportions copulating each day. The uterine contents of 76 were examined $13\frac{1}{2}$-$17\frac{1}{2}$ days postconception and a total of 869 corpora lutea counted. — In the absence of radiation, hypoxia had no effect on any factor studied. An unexpected result was the highly significant increase in corpora lutea from 10.5 per female in controls to 14.2 and 12.9 in air- and hypoxia-irradiated groups respectively. Within

the 5-day mating period, the excess increases with the interval between irradiation and copulation. Assuming the proportion of the corpora lutea count represented by fertilized eggs to be equal in all groups (assumption being tested), and assuming indirect effects of maternal irradiation on littersize negligible because of shielding, the data indicate that, with 400 r (in air), dominant lethals are induced in oocytes with a frequency approximately equal to that induced in sperm by the same dose. In contrast to sperm results (W. L. Russell et al., Genetics 36: 574), hypoxia probably provides some protection to oocytes.

RUSSELL, W. L., L. B. RUSSELL, J. S. GOWER and C. W. SHEPPARD, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Neutron-induced dominant lethals in the mouse. — Young adult male mice were exposed, four at a time, in a small lead chamber with 2-in. wall thickness to fast neutrons from a beryllium target placed in the proton beam of the Oak Ridge 86-in. cyclotron. The Victoreen dosimeter used was calibrated against three tissue-equivalent ion chambers. Gamma-ray contamination was estimated by a bismuth ion chamber to be about 10% of the total dose in rep. On the day following exposure each male was placed with four non-irradiated females. At a late stage of pregnancy in females that had mated from two to six days after irradiation of the males, the uterine contents were examined and the corpora lutea counted. Eighty-seven pregnancies (846 corpora lutea) from males exposed to 14 doses ranging from 47 to 369 rep, and 20 pregnancies (192 corpora lutea) from control males, were examined. The results show that, as with X-ray-induced dominant lethals, the deaths occur both before and shortly after implantation. There is no significant increase in mortality in later stages of gestation. Survival is 50% of that in the controls following a dose of about 100 rep. The relative biological efficiency of fast neutrons compared with X rays at this level of effect is about 8. — Dominant lethality in embryos from later matings (19 to 26 days after exposure) was determined for two doses and, like that from X rays, was found to be much greater than for the early matings.

ST. AMAND, W., University of Tennessee, Knoxville, Tenn. Differential frequency of acentric fragments induced in grasshopper neuroblasts by X-irradiation at known mitotic stages. — The x-ray sensitivities of the stages of mitosis were determined in hanging-drop preparations of neuroblasts of the grasshopper, Chortophaga viridifasciata (DeGeer), at 38 ± 0.5°C. Cells were mapped and identified as to stage of mitosis just before treatment with 32 r of X-rays. The mapped cells were examined immediately after treatment and then re-examined at short time intervals for as long as eight hours to detect acentric fragments as the cells passed through subsequent anaphases. All determinations included in this report were made in living, unstained cells at the first anaphase following treatment. — The highest frequencies of fragments are found in cells irradiated in middle and late anaphase and telophase. The sensitivity of cells in interphase at the time of treatment is slightly lower than that of cells irradiated in early prophase. The numbers of fragments induced decreases from early

to late prophase and reaches a minimum in cells irradiated in prometaphase. During metaphase and early anaphase sensitivity increases to reach the anaphase-telophase peak. (AEC Predoctoral Fellow.)

SCHULTZ, JACK and D. A. HUNGERFORD, Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia 11, Pa. Characteristics of Pairing in the Salivary Gland Chromosomes of Drosophila melanogaster. — Improved squash preparations, using silicone-coated slides and cover slips, have provided material for a detailed analysis of the pairing in the salivary gland chromosomes. The pattern of pairing was specified by the frequency, localization and length of unpaired regions. These were defined by the section numbers of Bridges' salivary gland chromosome maps: midpoint giving localization, number of sections giving length. In the X chromosome a sharp mode of midpoints lies in the central chromosome region; lengths have a bimodal distribution, intermediate classes being rare. Frequencies of failures are characteristic for each chromosome, 2L being notably low. Autosomal inversions do not change the frequency of failures of pairing in the X; however, the frequency of short failures of pairing does increase with inversions present in both autosomes. — Triploids and X-trisomics were also studied. Chromosomes were associated by threes, with frequent failures of pairing between one chromosome and the other two. The trisomic X's were like the triploids; but the frequency of pairing failure is increased by autosomal inversions. — Pairing failures in different chromosome arms appear uncorrelated in frequency, excepting the two arms of an autosome in the triploid. — The data are consistent with a picture of pairing restricted in time, and zippering from both chromosome end and centromere. The interchromosomal influences described have bearing on interchromosomal effects on crossing over at meiosis.

SCHWARTZ, D., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Studies on the mechanism of crossing over. — A mechanism of crossing over is suggested which incorporates the Belling scheme and sister-strand crossing over. On this hypothesis chromosomal replication occurs by the formation of a new chromatid on a template which is provided by the parent chromosome. During the process of replication crossing over occurs only between the two new chromatids of homologous chromosomes. A three-strand double results from a double crossover where a sister-strand exchange occurs in one of the chromosomes between the non-sister crossovers. Similarly, a four-strand double results from a sister-strand exchange in each of the chromosomes. Experiments were conducted on somatic crossing over between attached-X chromosomes in D. melanogaster heterozygous for y and sn (y · sn). Since there is little or no sister-strand crossing over in somatic tissue of Drosophila, different results would be obtained from somatic crossing over, depending on whether the exchange involved only the newly formed chromatids or old and new chromatids as well. Crossing over between an old and a new chromatid proximal to the sn locus should result in a twin spot, while crossing

over between the two new chromatids will give rise only to wild type tissue. The frequency of twin spots observed was extremely low. When the flies were subjected to the environmental conditions which increase the frequency of sister-strand exchange (Brown and Hannah, Proc. Nat. Acad. Sc., 38: 1952) a large increase in twin spotting was observed.

SHULL, GEORGE HARRISON, Princeton University, Princeton, New Jersey. Chloralbinism in the Burr Oak (Quercus macrocarpa (Michx.)) and its putative modification through grafting. — Over a period of 38 years during which I have resided at 60 Jefferson Road, Princeton, New Jersey, I have found an occasional albino seedling of one or other of two mature specimens of Burr Oak (Quercus macrocarpa (Michx.)) growing at western edge of this property. The crop of acorns has varied from year to year, but was fairly abundant on the northerly of the two trees in 1949 when I decided to test the assumption that this tree is a heterozygote for chloralbinism. I collected just over 1000 acorns and planted them in the nearby garden, noting that many of them were infested by weevil larvae. Later it was observed that the planting had been found by squirrels which had dug them quite systematically in places. In the spring of 1950 I was disappointed but not much surprised, to get only 52 seedlings from this planting of more than 1000 acorns. These acorns produced 43 typical green seedlings and 9 albinos, aproximately a 5 : 1 ratio, thus confirming my assumption that this tree, at least, is a heterozygote even though the ratio was unexpectedly "bad." In the hope of "improving" the ratio I decided to try again but the next two years were "off years," in which the crops of acorns were very meager. In 1952 both trees produced abundant crops of acorns, and with Mrs. Shull's help we harvested and planted 546 acorns from the southerly tree and 3,100 from the northerly tree. We were gratified to note the much less frequent occurrence of weevil larvae than in 1949, and we took precautions to protect the two seed-beds from marauding squirrels. In the spring of 1953 the acorns from the southerly tree produced 241 seedlings, all typical green, while those from the known heterozygous tree produced 1,945 green, 3 "Golden" mutants(?) and 15 albinos. This still smaller ratio of albinos (129 : 1) is amazing in view of the assuredly more adequate population. The explanation probably inheres in the fact that the oaks like many other monoecious species are largely self-incompatible, so that most of the effective pollen which produced acorns on this albino-producing tree came from the homozygous tree, thus hiding the presence of an albino gene by a dominant chlorophyll producer. — In the hope of establishing a parasitic relationship between the albinos and their green sibs each albino was set in an 8-inch pot in close association with a green sib; slices of bark were removed with a razor knife, from the adjacent sides of the two stems, which were then wrapped snugly together with raffia. In three of these grafted pairs the albino became detached from its acorn, and since it was unable to make any food materials without a supply of chlorophyll, the detached stems immediately died. One other died so quickly that I suspect that it also lost connection with its acorn. The eleven others lived on, producing fresh leaves presumably until the organic food stored in the

acorns by the mother was completely exhausted. Three still had one or two fresh leaves on October 1, but whether the grafting had any influence on the growth of the albino member of the pair is problematical. A most extraordinary observation was that two weeks or more after the grafting, the leaves of the albinos were no longer strictly albino, but were to varying degrees speckled, flaked or blotched with green. In one plant (No. 10) several leaves developed so much chlorophyll that they retained their full turgor, similar to the normal leaves of the green sibs. In all other cases the development of specks and blotches of green did not represent sufficient chlorophyll to noticeably affect the growth and decadence of the leaves, which remained thin and soon became moribund, developing brown patches as they starved to death, and dropped off. Unfortunately all fifteen of the albinos were used in grafting, thus leaving it problematical whether the grafting had anything to do with the production of obvious green tissues in the leaves of the "albino" trees, but I have seen somewhere near twenty albino seedlings in past years and never before noticed any green on them. I do not believe I could have overlooked it.

SIEGEL, A. L., I. RAPPAPORT, and S. G. WILDMAN, University of California, Los Angeles. Some natural relationships of strains of Tobacco Mosaic Virus. – Eight strains of TMV have been found to fall into four natural groups when the following properties were characterized: color, electrophoretic mobility, type of symptom of Nicotiana sylvestris, sensitivity to inactivation by ultraviolet light, and antigenic constitution. The strains which fall into a group are identical in all of these properties except for antigenic constitution. Slight serological differences were found to exist between the members of a group and very large differences between groups. Strains in the same group differ markedly in the symptoms that they produce in Nicotiana tabacum. Those strains which invade N. sylvestris systemically all have the same color, electrophoretic mobility, sensitivity to UV, and are almost identical serologically. Those strains which produce the nectrotic local lesion reaction in this host, differ from the above strains in one or more of these properties, indicating a correlation between the in vivo and in vitro properties of TMV. The establishment of a number of strains with several sorts of differences between them provides a series of markers to test for the possibility that recombination takes place among the plant viruses.

SILVERS, WILLYS K., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and Department of Zoology, University of Chicago, Illinois. Histological distinction between hair follicles of albino and spotted genotypes of the mouse. – The hair follicles of white spots produced by genes at seven different loci (bt,sp,va,s,fl, Mi^{wh} and W) of the mouse were investigated histologically. All present the same picture of an absence of pigment cells. In contrast to this the hair follicles of albino animals contain large hyalinated cells distributed along the matrix which have been shown to be "pigmentless pigment cells." The identity of these cells have been confirmed by observing similar cells, with pigment granules in their

cytoplasm, in lightly colored genotypes ($c^{ch}c^{ch}pp$). Animals of the genotype $AYac^{e}c^{e}$, although phenogypically like $W^{v}W^{v}$ genotypes (i.e. white with black eyes, exhibit "Pigmentless pigment cells" whereas the latter is histologically "one big spot." The "environmental" spotting found in Brown cd's is histologically similar to that produced by proven spotting genes. In addition it has been noted that both "dd" and "lnln" genotypes exhibit a morphologically "abnormal" melanocyte in that here there is a "clumping" of granules within the pigment cell.

SOKAL, ROBERT R. and PRESTON E. HUNTER, University of Kansas, Lawrence, Kansas. Selection for correlated quantitative characters in Drosophila. — Correlated responses occur frequently during selection experiments designed to throw light on the nature of quantitative inheritance. Analysis of such correlations may help resolve some of the conflicting views recently expressed on the genic mechanism underlying quantitative characters. — Two strains of D. melanogaster have been selected for resistance and susceptibility, respectively, to DDT in the nutrient medium. The experiment is in its 33rd generation. The resistant strain has attained a level of tolerance approximately 2.5 times that of the control strain, while the control strain is approximately 1.7 times as tolerant as the susceptible strain. — Pupation site appeared early as a correlated character. Resistant flies pupated increasingly at the margin even in nontoxic medium vials, while susceptible flies pupated predominantly away from the margin. Differences in % peripheral pupation between the two strains attained approximately 20%. — At a later date two new strains were selected for peripheral vs. central pupation, respectively. These strains are now in their 13th generation. They have attained differences of approximately 40% in peripheral pupation. When tested for differences in DDT resistance the peripherally pupating strain was up to 1.7 times as resistant as the centrally pupating one. Thus selection for resistance brings changes in pupation site, while selection for pupation site differences brings about resistance differences. — A discussion of this and other evidence suggests that the observed phenomena are due to spurious pleiotropism sensu Grüneberg.

SONNENBLICK, B. P., Rutgers University, Newark, N. J., and the Marine Biological Laboratory, Woods Hole, Massachusetts. Neotetrazolium as a visible indicator of radiation damage: Reaction of Allium cytoplasm to x-radiation. — To study the response of biological systems to radiation, investigators have employed total-cell and various partial-cell techniques to separate nuclear and cytoplasmic effects. We have been observing the visible response of untreated and x-radiated root tip cells to neotetrazolium chloride. Upon reduction, this salt undergoes progressive color changes becoming converted to insoluble purple-black granules located characteristically in the perinuclear cytoplasm, with no visible evidence of intranuclear reduction. This is essentially a response of cytoplasm which can be utilized to demonstrate the spatial and temporal patterns of reducing enzyme systems (dehydrogenases). Active sulfhydryl groups have

a role in mediating the reaction. — Doses to 18,000r (delivered at 600r/min.) were received by roots of germinating bulbs, treatment exceedingly detrimental to nuclei and subsequent root growth, while excised tips received up to 480,000r (at 6000r/min.). For 2-3 days after exposure (18,000r), growing root meristems showed the color reaction with the intensity and speed of controls. Reduction of the salt falls off by 4-5 days postradiation, but the reaction is still evident 20 days after x-raying. Excised roots given 240,000r could reduce neotetrazolium as fast and as intensely as controls, but other tips tested 4 and 24 hours after treatment gave a diminished reaction. Only with 480,000r was an effect on the indicator apparent in roots tested within a few minutes postradiation. 48 hours after this massive dose cells could still reduce the dye but with decidedly lessened ability. — Damaging x-radiation (many times above lethality) does not prevent neotetrazolium reduction in Allium cytoplasm.

STONE, W. H. and M. R. IRWIN, Department of Genetics, University of Wisconsin, Madison, Wisconsin. Immunogenetic studies on the J character of cattle blood groups. — The antigenic character of the erythrocytes of cattle called J is detectable only by means of interaction with normal antibodies from the serum of cattle lacking the factor. — Recent observations show that a soluble substance with serological activity specific for the J character may occur in the serum of cattle whose cells may or may not possess the character. An extensive analysis has shown that the bloods of cattle may be divided into at least three major phenotypic classes: (a) J^{CS}—those with J substance on the cells and in the serum, (b) J^{S}—those with J substance in the serum only, and (c) j^{a}—those lacking demonstrable J substance. Individuals in this last class are the potential possessors of the normal J antibodies. — The distribution of the three phenotypes in the offspring from different kinds of matings provided evidence consistent with the hypothesis that these characters are controlled by a series of allelic genes arranged in a descending order of dominance, i.e. J^{CS}, J^{S} and j^{a}. Further, the concentration of J substance in the serum of J^{CS} individuals was usually significantly higher than that of J^{S} individuals. These data suggest that the concentration of soluble J substance determines, in large part, the reactivity of the cells. The present hypothesis is that when the concentration of J substance is above a definite threshold, the cells may acquire enough J substance to become reactive with J antibodies. Consequently, the genes J^{CS} and J^{S} are examples of allelic genes with different quantitative effects.

STONE, W. H. and W. J. MILLER, Department of Genetics, University of Wisconsin, Madison, Wisconsin. A New Normal Antibody of Cattle Serum. — A normal antibody of cattle serum has been discovered which detects the antigenic factor of cattle erythrocytes called U_2. Previously, the U_2 factor has been detected only by means of isoimmune sera. The standard reagent (typing fluid) containing a single specificity for the factor U_1 (U_1-reagent) has been obtained routinely by absorbing anti-U_1 isoimmune serum with erythrocytes containing the factor U_2. The standard U_2-reagent

has been an unabsorbed anti-U_1 isoimmune serum containing specificities for both of the factors U_1 and U_2. Thus, the standard U_1-reagent reacts only with cells containing the factor U_1, while the standard U_2-reagent reacts with cells containing either or both of the factors U_1 and U_2. — This new normally occurring antibody reacts as a U_2-reagent, but is unique from the isoimmune U_2-reagent because it contains but a single specificity for the factor U_2. Thus, this new normal antibody reacts only with cells containing U_2 and consequently is useful in distinguishing cells containing U_1 alone from those containing both U_1 and U_2. — Using the standard U_1-reagent and this new normally occurring U_2-reagent, it is possible to distinguish cells of individuals which are heterozygous for the causative genes (U_1U_2) from those of either homozygotes (U_1U_1 and U_2U_2). — A distinctive characteristic of this normal antibody is that it fails to cause hemolysis of cells containing the factor when used at high concentrations, but causes complete hemolysis at high dilutions. This may explain why this antibody was not discovered previously.

SRB, A. M., Cornell University, Ithaca, N. Y. Shifts in heterocaryotic dominance relations effected by modifier genes in Neurospora. — Arginineless and lysineless mutants of Neurospora have been obtained on identical genetic backgrounds by treating wild-type conidia with ultraviolet light and utilizing the technique of filtration followed by selective plating. Growth of arginineless mutants is inhibited by lysine, and lysineless mutants are sensitive to arginine. Heterocaryons between an arginineless and lysineless strain occasionally grow on minimal agar medium at approximately the rate of wild type. When conidia from such heterocaryons are crossed onto wild type, the relative frequencies of ascospores of the two mutant types indicate that wild-type growth rate is achieved only when precise mutant nuclear ratios are maintained. — By plating irradiated conidia of an arginineless strain onto medium supplemented with arginine plus an inhibitory concentration of lysine, arginineless strains relatively insensitive to lysine can be selected. Analogous procedures permit the recovery of lysineless strains relatively insensitive to arginine. In either instance, an alteration in amino acid sensitivity has been identified with mutation at a single gene locus. — Heterocaryons between arginineless and lysineless strains carying these modifier genes almost always give wild-type growth rate on minimal agar. Estimates of nuclear ratios, based on the procedure utilized with the original mutant strains, indicate that wild-type growth rate may occur under fairly wide latitude of nuclear proportions. Although the method of estimating nuclear ratios is in certain respects unsatisfactory, the results indicate that heterocaryotic dominance relations among the two biochemical mutant genes and their wild-type alleles have been altered by modifying genes.

STALLWORTHY, W. B. and R. H. BUKER, (Introduced by B. R. Speicher), University of Maine, Orono, Me. Respiration of Habrobracon juglandis (Ashmead) for various sex types. — Oxygen uptake of individual wasps was followed in a modified Gerard-Hartline respirometer. The

transit of the meniscus was timed across the micrometer eyepiece of a low power microscope. Temperature was held at 5.0°C. to eliminate spontaneous activity. Animals were weighed separately to ± 0.1 mg. — Test animals, bred from "pectinophorae" strain of Habrobracon carrying the recessive eye colour gene, cantaloup (c), at 30°C., were used within 24 hours of eclosure. From cultures of this strain, and from the cross of c/c ♀♀ X + ♂♂, were obtained: (1) Haploid males (c), (2) Diploid females (c/c), (3) Diploid males (c/+). The last, when successfully mated to c/c ♀♀ sometimes produced a few (4) Triploid females (c/c/+). — Respiration measurements on 20 individuals of each type gave the following averages in cubic millimeters of oxygen per milligram per hour; haploid males—0.550 ± 0.021; diploid females—0.540 ± 0.015; triploid females—0.601 ± 0.026. These three do not differ significantly from one another. The O_2 uptake of the diploid males (0.790 ± 0.041) was greater than that of the other three types and well beyond the 1% level of confidence. Two subsequent replications reproduced this difference at the 5% level or better, though the absolute values were slightly lower than in the first series. The diploid males also showed throughout a higher variability than the other types. (This work was assisted by a grant from the Coe Research Fund, University of Maine.)

STRAUSS, BERNARD S., Department of Zoology, Syracuse University, Syracuse 10, New York. Gene Interaction: The mode of action of the suppressor of acetate-requiring mutants of Neurospora crassa. — Certain single-gene, acetate-requiring mutants of Neurospora crassa (ac) are deficient in their ability to oxidize pyruvate directly. As a result of the ac mutations, 3-hydroxy-2-butanone (acetylmethylcarbinol) accumulates. ac mutants can decarboxylate pyruvate to acetaldehyde and can form ethanol. The growth characteristics of the ac mutants indicate that they can form acetate from glucose or ethanol. — ac mutants are inhibited by glucose or sucrose. This glucose inhibition is relieved by the single-gene suppressor mutations sp and car, which lower the activity of pyruvic carboxylase, reduce the amount of ethanol formed, and permit some growth of ac sp and ac car strains in the absence of added acetate. The sp mutation also lowers alcohol-dehydrogenase activity, but this activity can be partially restored by growth of sp strains in the presence of ethanol. — ac^+ car strains metabolize glucose by pathways which produce more of the initial respiratory CO_2 from the C-1 carbon of glucose in contrast to ac^+ sp strains, which behave like wild-type. However, as a result of a nearly complete block in pyruvate metabolism both ac sp and ac car produce more of their respiratory CO_2 from the C-1 carbon of glucose. Consequently less of this C-1 carbon finds its way into the ethanol formed by ac sp and ac car compared to wild-type and ac sp^+. — It is supposed that as a result of the ac mutations, acetaldehyde (or some closely related compound) is formed from glucose in amounts inhibitory to the ac mutants. Retarding acetaldehyde formation by either suppressor mutation relieves glucose inhibition and permits some growth in the absence of acetate. — Genetic suppressor mutations may often be "loss" mutations which prevent the formation or accumulation of inhibitory substances formed as a result of a primary

mutation. (Supported by contract AT(30-1)1138 between Syracuse University and the Atomic Energy Commission.)

STURTEVANT, F. M., G. D. Searle & Co., Chicago, Ill. Artificial selection program for degenerative diseases in rats. — Six males and 6 females each of Sprague-Dawley, Sherman, Wistar, and wild Norway rats were doubly reciprocally crossed, with the exception of the wild females, which never became pregnant. The resultant colony was inbred to obtain as variable an F_1 as possible from which to begin selection for spontaneous (a) essential hypertension, and (b) diabetes mellitus. At a given age, blood pressures were estimated using the photoelectric tensometer, diabetic tendencies by the intraperitoneal glucose tolerance test. In each subsequent generation, 10% of the males were mated randomly to 20% of the females, always using those animals at the most hypertensive or diabetic tail of the distribution curve. Exceptional individuals are progeny-tested and back-cross, where possible. — From an original F_1 of 436 rats, 2 lines have been established which average 200 rats/generation. Two generations of selection for hypertension have not altered the mean (123 mm Hg), but have significantly decreased the variance from 25 to 17 ($P < 0.05$). — In the diabetic line, one generation of selection significantly increased the mean from 120 to 186 mg % at 2 hours ($P < 0.01$) and the variance from 800 to 1740 ($P < 0.01$). — Selection in these 2 lines is to continue over many generations, with the rates of evolution and increasing homozygosity being gauged by the increases of the mean and decreases of the variance, respectively.

THEILER, KARL, Anatomisches Institut der Universitaet Zuerich and Roscoe B. Jackson Lab., Bar Harbor, Maine. The development of split vertebrae in the Short Danforth mouse. — The occurrence of split vertebrae in homozygous Sd-mice was observed in 1943 by Gluecksohn-Schoenheimer. Embryological analysis of this manifestation on the CFW-background shows that the origin can be traced back to the mesenchymous stage. The migration of the bilateral anlagen towards the midline is delayed. Consequently at chondrification cartilaginous centers develop separately on each side of the body. In the posterior region a cleft regularly begins at L_2/L_3 and gradually becomes wider caudally. In the cervical region approximately $\frac{1}{2}$ of the Sd/Sd embryos showed less marked clefts affecting C_2/C_3. Along the whole vertebral column, the dorso-ventral diameter of the vertebral bodies is reduced to $\frac{1}{2}$ normal size both in newborns and in 14-day embryos. The spinal cord is not split and the arches are well developed. This singular effect on the bodies may be due to the precipitous degeneration of the notochord.

TOMES, M. L. and F. W. QUACKENBUSH, Purdue University, Lafayette, Indiana. Two factor interaction governing beta-carotene content in the tomato. — In the tomato the gene B governs the formation of high concentrations of beta-carotene. The gene was originally designated as an incomplete dominant (Lincoln and Porter 1950). Advanced generation studies

indicate that the gene is dominant as suggested by Tomes, et. al. 1953. In a cross of a high lycopene, red strain (Rutgers) x a true-breeding, high beta, orange strain the intermediate nature of the F_1, as well as the F_2 segregation can be explained by the action of a dominant inhibitor of B contributed by the red parent. The F_2 ratio on this basis should be 4 red (high lycopene, $bbi^{B}i^{B}$ or bbI^{B}-): 9 intermediate ($B\text{-}I^{B}$-): 3 orange (high beta, $B\text{-}i^{B}i^{B}$). The expected F_3 progenies of the above F_2 genotypes have been derived. For example, high beta-carotene phenotypes (85 to 95% of total pigment, beta-carotene) may either breed true for high beta content or yield 3 orange: 1 red. The red genotype ($bbi^{B}i^{B}$), from such a 3 orange: 1 red progeny, when crossed with true-breeding, high-beta strains produces an F_1 containing 85 to 95% beta-carotene, demonstrating the dominance of B in the absence of the inhibitor. Preliminary data indicate that red strains lacking the inhibitor produce slightly more beta-carotene than do types such as Rutgers. Thus four levels of provitamin A content are available in genotypes homozygous for the two factors.

TRASLER, DAPHNE G., and J. D. METRAKOS, McGill University, Montreal, Canada. Sex ratio of offspring from irradiated male mice. — Preliminary experiments performed at this laboratory by Kalmus, Metrakos and Silverberg (Science 116: 274-275; 1952) suggested that the sex ratio of offspring from irradiated male mice of the A/HeJax strain (1.53) was significantly higher than that of the control group (1.01), and that this change was due almost entirely to a significant reduction of females in the experimental group. Employing the same procedures, experiments were undertaken in order to check these observations and also to ascertain whether the same effect could be obtained in other strains. In the three strains used there was: 1) no significant change in the sex ratio of the DBA/1 Jax strain; 2) a significant reduction in the number of males but not of females in both the C57BL/Fr and A/Jax strains. The present results, therefore, are, on the whole, contrary to those of the earlier experiments, and cannot be explained except by recourse to the capricious nature of sex ratios in mice. (Work supported by a grant-in-aid from the National Research Council of Canada).

VOGEL, HENRY J., Yale University, New Haven, Conn. On the formation of the biosynthetic enzyme acetylornithinase. — Mutant strain 39A-23 of Escherichia coli responds alternatively to ornithine, citrulline, arginine, or to their biosynthetic precursor alpha-N-acetylornithine. The latter is converted to ornithine by the enzyme acetylornithinase. Strain 39A-23 gives diphasic growth on a mixed supplement of a growth-limiting amount of L-arginine and an excess of alpha-N-acetyl-L-ornithine: exponential first-phase growth at wild-type rate with preferential utilization of arginine, followed after an abrupt break by slower exponential second-phase growth on acetylornithine, the second-phase rate decreasing with increasing initial arginine concentration. Addition of ornithine during second-phase causes immediate resumption of wild-type growth rate. Upon cessation of growth on limiting arginine as sole supplement, addition of

acetylornithine results in adaptation, after a lag, to that growth rate expected as second-phase rate, had the acetylornithine been added together with the arginine. These growth experiments and assays of acetylornithinase extracted from cells in relevant growth phases suggest that: (1) the biosynthetic enzyme acetylornithinase can be formed adaptively in response to its substrate; (2) the adaptive phenomenon is resolvable into two elements, (a) a variable enzyme-forming capacity and (b) enzyme formation, both elements being substrate-dependent; (3) during growth on arginine in the presence of acetylornithine, capacity as well as acetylornithinase formation are antagonized; (4) the variation of capacity with cultural conditions may reflect differences in the sites of enzyme formation with respect to either their number per cell, or their functioning, or both. — The results are consistent with the view that enzyme formation is controllable through mechanisms affecting the propagation or functioning of enzyme-forming sites.

VOLPE, E. PETER, Newcomb College of Tulane University, New Orleans, La. Intensity of isolation between Bufo americanus and Bufo fowleri from different geographical regions. — Previous work on interspecific hybrids between Bufo americanus and Bufo fowleri by Blair (1941) and Volpe (1952) demonstrated that the cross is highly successful and viable hybrids result. Some developmental abnormalities were found in a few of the crosses made, but it was difficult to determine whether the per cent abnormal was significant or merely represented the usual encumbrances encountered in rearing amphibian embryos. In the present investigation, different geographical representatives of each species have been hybridized. Mating pairs were obtained from areas where both species are sympatric (where a danger of hybridization exists) as well as outside of the distributional overlap of each species. Special attention was paid to the developmental rates of the hybrid embryos, since deviation from the control rate (i.e., normal cross) is a sensitive indicator of any ill effects due to hybridization. The per cent hybrid abnormalities and the amount of deviation of hybrid developmental rate from the normal rate were least in crosses involving B. fowleri derived from the geographical regions in which, or close to which, B. americanus occurs. There is no indication of hybrid failure in regions where the two toad species are sympatric. On the contrary, the greatest degree of hybrid divergence in developmental rate from the normal was encountered in crosses among allopatric representatives of the two species. Hybrids derived from the cross, Louisiana B. fowleri ♀ x B. americanus ♂ from North Carolina or Pennsylvania showed structural abnormalities and a marked retardation in developmental rate. Apparently, geographical separation has served to allow the development and retention of physiological distinctions.

WAINWRIGHT, S. D., Atomic Energy of Canada Ltd., Chalk River, Ontario. Effects of post-irradiation treatment with iodoacetate upon ultra-violet irradiated spores of Streptomyces T12. — A short period of incubation of ultra-violet irradiated spores of Streptomyces T12 with iodoace-

tate effects an enhancement of survival associated with a change in the proportion of cells giving rise to colonies exhibiting heritable variations of character. The presence of peptone suppresses the enhancement of survival and affects, but does not prevent, changes in the proportion of variant colonies. Studies of these and related phenomena are to be reported. (National Research Council (Canada) Fellow 1952-3. Atomic Energy of Canada Ltd. Fellow 1953-4.)

WALLBRUNN, H. M., University of Florida, Gainesville, Florida. Tyrosinase and tyrosinase inhibitor in Betta splendens. – In the fighting fish cc inhibits the production of black on the body and fins. C is completely dominant. In C-bb fish the number of melanophores on the body is greatly reduced and all but eliminated on the fins. B is dominant and cc is epistatic to B. The three phenotypes mentioned are, therefore, cc (called cambodia), C-B- (dark red), and C-bb (bright red). It is also possible to obtain cc fish with colorless rather than red fins due to other genes. – Homogenates of ground fins and distilled water were mixed with tyrosine and tested for tyrosinase activity in a Warburg respirometer. Net oxygen uptake and color of flask contents was used as a measure of tyrosinase activity. – Cambodia with colorless fins showed no activity. Cambodia with red fins and C-bb both had tyrosinase but took up much less oxygen and produced less black than C-B-. – Iodoacetamide was added to inhibit inhibitors of tyrosinase. When added to cc and C-B- the curves of oxygen uptake are approximately the same, indicating that cc fish have more inhibitor rather than less enzyme than C fish. Evidence points to the inhibitor acting on a step subsequent to the oxidation of tyrosine.

WALSH, M. P. and J. B. MC MAHON, Boston College, Chestnut Hill 67, Mass. The Cytological Effects of OMPA. – Because of its anticholinesterase properties, octamethyl pyrophosphoramide (OMPA) has been used with varying degrees of success as an insecticide and as a chemotherapeutic agent in the treatment of myasthenia gravis. The purpose of this investigation was to determine the exact cytological effects produced by OMPA and to compare these cytological findings with those produced by other chemicals. Concentrations of OMPA ranging from 0.1% to 0.00025% were used on onion root tips. The tips were cut at various time intervals. OMPA (0.01%) produced stickiness, chromosome bridges, star-metaphases, apolar-c-anaphases, pycnotic kinetochores, chromosome constrictions and fragmentations, and chromosomes that resembled lamp-brush chromosomes. Weaker concentrations produced the typical c-mitotic effects. Binucleate cells, multipolar spindles and polyploid nuclei were observed during recovery periods. It seems probable, therefore, that OMPA has an effect not only on the spindle mechanism as c-mitotic chemicals generally have but also a dissolving action on the matrix of the chromosomes. Some of the results of the present investigation would seem to indicate that OMPA may also be a mutagenic agent.

WALTERS, J. L. University of California, Santa Barbara. Spontaneous chromosome breakage in meiosis of Paeonia californica. – A spectrum of

fragmentation occurs ranging from single fragments to extreme breakage of the entire chromosome complement; the fragments vary greatly in size. Two-chromatid fragments predominate at first metaphase, the stage at which fragments first appear; one-chromatid fragments are more common at later stages. The extent of breakage varies considerably from cell to cell within an anther as well as from plant to plant. Inversion heterozygosity is frequent in this species, but many more fragments occur than can be attributed to inversion crossing-over; most fragments are unaccompanied by bridges. The species also shows a complete series of translocation heterozygotes; there appears to be no correlation between the extent of spontaneous fragmentation and the degree of translocation heterozygosity, nor does fragmentation exhibit the geographic regularities previously reported for the translocations. The total picture of meiotic irregularities occurring in this species is perhaps the most extensive yet found in a natural population.

WALTERS, MARTA SHERMAN, Santa Barbara Botanic Garden and University of California, Santa Barbara. Radiomimetic chromosome aberrations in species and interspecific hybrids of Bromus. — The following radiomimetic phenomena have been observed in certain species and interspecific hybrids of Bromus: (1) chromosome breakage and reunion; (2) centromere misdivision; (3) chromosome erosion; (4) abnormal chromosome coiling; (5) pseudobivalents at first metaphase; (6) difficulty of separation at first anaphase; (7) chromosome stickiness. The occurrence and extent of these radiomimetic effects are variable among the different species and hybrids studied. The species and hybrids are grown at some distance from their normal environment. Thus the aberrations may result from changes in the environment of the species, or from interactions of the two genomes in the interspecific hybrids. It is believed that the chromosomes may respond in similar ways to many different stimuli.

WEIR, J. A., University of Kansas, Lawrence, Kansas. Influence of the male on sex-ratio of offspring in high and low blood-pH lines of mice. — Selection for variations in blood-pH was successful in one generation in fractionating an outbred line of mice into a high pH line (blood pH 7.466 ± .0048) and a low pH line (pH 7.420 ± .0051). Concomitant deviations in sex-ratios, from the outbred line (blood-pH 7.458 ± .0044, sex-ratio 49.8% ♂♂), were at once obtained. The first generation of high blood-pH mice, at birth, consisted of 52.8% ♂♂, the low blood-pH group of 42.8% ♂♂. The significant differences in both blood-pH and sex-ratio have remained stable through 9 subsequent generations of inbreeding. A possible effect of pH of cervical mucus on sex-ratio has been postulated (J Hered. 44:133-138, 1953). — Preliminary results from reciprocal crosses now point to a ♂ influence. High pH ♂♂ x low pH ♀♀ produced 65.0 % ♂♂ (10 litters). Low pH ♂♂ x high pH ♀♀ produced 43.3 % ♂♂ (12 litters). — A sample of the extensive Iowa State College data gave concordant results. Sex-ratios in high blood-pH inbred strains were: S, 51.3% ♂♂ ; R.I.,50.6% ♂♂ . Ratios in low pH strains were: Z, 47.7% ♂♂; L, 46.8% ♂♂; Ba, 45.9% ♂♂ .

Limited data for reciprocal crosses have indicated the expected type of ♂ parent effect. The physiological basis for these findings has not yet been worked out. — There is no difference between sexes in blood-pH in any of the mouse strains. (Supported by grant (G-3141) from the National Institute of Health, U. S. Public Health Service.)

WHITING, ANNA R., University of Pennsylvania, Philadelphia, Pennsylvania and Oak Ridge National Laboratory, Oak Ridge, Tennessee. Frequencies of dominant and recessive lethals induced in Habrobracon eggs by X rays in air and in nitrogen. — Eggs X-rayed in late metaphase 1 show high rate of terminal deletions correlated with dominant lethal effects. Recessive lethal rate is low. Chromosomes in this stage appear to be under tension and are not in contact. Dominant lethal rate may represent total original breakage because of tension while recessive lethals may result from minute changes within chromosomes. — Eggs (n) from irradiated unmated females which hatch have no lethals. Surviving daughters (2n) from females mated to untreated males, when bred unmated, can be classified on basis of hatchability of their eggs into those with no recessive lethals (100% hatchability), those with one (50% hatchability), etc. Majority of lethals act before hatching. — Unmated females were exposed to 1100 r in a current of air or of nitrogen, the latter during irradiation only, about three minutes. Hatchability of metaphase 1 eggs of exposed females is, for unmated, in air 12.56%, in nitrogen 54.65% ($\sigma_{diff.} = 2.27$); for mated, in air 20.99%, in nitrogen 61.08% ($\sigma_{diff.} = 1.82$). Estimated dominant lethal rates are 74.82% for air, 35.76% for nitrogen. 15.12% of daughters of females irradiated in air carried recessive lethals (Dr. Gertrude Heidenthal reports 14.2% for 1000 r), 5.35% of those irradiated in nitrogen ($\sigma_{diff.} = 3.12$). 5.75% of former carried more than one recessive lethal, 0.95% of the latter. Biologically equivalent dose in air for nitrogen data is about 400 r. The change in rates of both dominant and recessive lethals under conditions of this experiment suggests that there is a decrease in breakage in nitrogen. (This investigation was supported in part by a research grant PHS C-741(C4) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.)

WHITTINGHILL, MAURICE and ROBERT M. JOHNS, Department of Zoology, University of North Carolina, Chapel Hill, N. C. On the relation between X-ray-induced crossing over and Mendelian segregation in Drosophila. — During the course of experiments aimed at avoiding the full operation of Mendel's first law, evidence was obtained which fixed the time at which irradiation-induced crossing over had occurred. Males of D. melanogaster were irradiated with a 3000 r dose of X-rays at 35°C and testcrossed repeatedly for 18 or more days. Crossovers increased in frequency up through four successive subcultures, and non-crossover distribution changed at a slower rate toward more wild type offspring. Out of 24 possible chance orders of wild types among non-crossovers the one predictable order was realized. Significantly large differences were found between the 13th-18th day cultures and earlier cultures, both in mono-

hybrid segregation among non-crossovers and in the frequency of crossovers. Furthermore one male out of 19 given the treatment produced ratios which changed from the Mendelian 50:50 to 60 wild types versus 40 of Curly wings consistently and predictably. This ratio is very unlikely on the assumption of early (gonial) chromosome breakage and later (meiotic) crossing over, but it is perfectly predictable assuming the completion of crossing over in the gonial cell hit. Genetically dissimilar "twin spots" would be produced in the gonad by segregation of one crossover and one non-crossover into a fully viable daughter cell, and of the complementary crossover and non-crossover, each bearing lethals, into another cell. If the later cell were killed by homozygosis or its multiplication retarded, it would simultaneously explain the direction of change of complementary crossovers and the degree of asymmetry of complementary non-crossover offspring. (Work aided by a research grant from the U. S. Atomic Energy Commission.)

WOLFF, S., Oak Ridge National Laboratory, Oak Ridge, Tennessee and Biological Laboratories, Harvard University, Cambridge, Massachusetts. Effects of chemical protective agents on the restitution rate of radiation-induced chromosomal aberrations in Vicia faba. — Seeds of Vicia faba were presoaked in water for 24 hours and then irradiated with 600 r of X rays. Pretreatment of the seed with 2×10^{-3}M solutions of BAL or of sodium hydrosulphite decreased the number of chromosomal aberrations. Fractionation of the dosage into three 200 r doses with one hour rest periods did not decrease the number of two-hit aberrations except in the presence of BAL. This is interpreted as indicating that the BAL increases the restitution rate of the broken ends (in press). — To get further information on this phenomenon, 600 r was administered at varying intensities from 200 r/min to 3.3 r/min. There was no decrease in the number of two-hit aberrations except at the very lowest intensities, indicating that there is little or no restitution of broken ends occurring within the first two hours. If the seeds are pretreated with BAL there is an immediate reduction of two-hit aberrations as the intensity of the radiation is reduced. The BAL therefore causes restitution to occur sooner and consequently decreases the two-hour lag.

YANOFSKY, CHARLES, Yale University, New Haven, Conn. Further studies with the td mutants of Neurospora. — In a previous investigation of two tryptophan-dependent Neurospora mutants (td_1 and td_2) it was found that the tryptophan requirement characteristic of each strain was the result of a single genetic change. It was also reported that although the mutations appeared to be at the same locus, a suppressor gene (su_2) suppressed one strain (td_2) but not the other (td_1). — These studies have now been extended to include 23 additional mutants which are similar to td_1 and td_2 biochemically; all have a tryptophan requirement which cannot be satisfied by indole, and all those which have been examined (td_1-td_{22}) lack tryptophan synthetase (desmolase) activity. — The su_2 gene has been crossed into 22 of the 25 strains (all except td_{11}, td_{23}, and td_{24})

and, of these, it suppresses only td_2. A second non-allelic suppressor gene, su_6, obtained in strain td_6, has been crossed into 13 of the mutants (not td_{24}) and of these it suppresses only td_2 and td_6. A third suppressor gene, su_{24}, does not suppress either td_2 or td_6 but does suppress td_{24}. — Tryptophan synthetase activity has been detected in the suppressed mutants which have been examined, however in no case does enzyme activity approach the wild-type level. The tryptophan synthetase obtained from several of the suppressed mutants appears to be identical with the wild-type enzyme. — The results obtained emphasize the complexity of genic interactions in enzyme formation.

YAO, T. S. and ORSON N. EATON, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C. Heterosis in the birth weight and slaughter weight of rabbits. — The birth weight and the slaughter weight at 180 days of age for 915 rabbits, of four inbred strains and their F_1, F_2 and backcross generations, raised at the Agricultural Research Center, Beltsville, Maryland, were studied for heterosis in the crossbred generations. The growth rates of the inbreds and the crossbreds were compared and the crossbreds were found all significantly heavier than the inbreds after 90 days. The birth weight and slaughter weight of the F_1 generation were uniform and had the smallest variability while those of the F_2 generation had the largest variability. Significant heterosis for birth weight was found in five of the eight F_1 groups and in one of the four backcross groups. There was no heterosis for birth weight in the F_2 generation. All the crossbreds showed significant heterosis in slaughter weight. Maternal and paternal influences on heterosis were observed. Maternal influence on heterosis was found to be higher than the paternal influence in most cases. The higher maternal influence was interpreted as the results of the interaction between plasmagenes and chromogenes on growth heterosis. The results of previous workers on rabbit size inheritance and the material used are discussed. Lack of heterosis in their results was presumed to have some connection with the number of quantitative genes involved, the incompatibility between the allelic genes or the incompatibility between genes from the paternal side and cytoplasm from the maternal side.

YERGANIAN, G., Boston University and Children's Cancer Research Foundation, Boston, Massachusetts. Evidence disclosing the autosomal nature of sex chromosomes previously reported in the Chinese hamster, Cricetulus griseus. — Pontecorvo (1943) referred to the longest metacentric in the spermatogonial compliment as the X chromosome in the male Chinese hamster. Matthey (1951, 1952) arrived at the same conclusion, in addition to designating the "smaller heteromorphic homologue" as the Y chromosome. Since 1952, the writer has been inbreeding this species for use in cytogenetic and carcinogenesis studies. It soon became apparent that the two longest metacentrics were not sex chromosomes as reported above. Evidence supporting this observation was provided by an X-Ray induced reciprocal translocation involving one of the disputed homologues. — A "ring of four" configuration, formed by the

1st and 11th pairs of chromosomes, and a separate, normal-appearing sex bivalent was observed. The translocated autosomes were easily identified in spermatogonial metaphase. If these metacentrics happened to be sex determinators, this particular rearrangement would have involved the heterochromatic (allocyclic) segment (s), thereby altering the size and appearance of the configuration. -- Additional studies on methylcholanthrene-induced fibrosarcomata in females have not as yet provided information concerning the identity of the X chromosome. In this species, the corresponding male and female mitotic homologues are morphologically similar. Consequently, the sex chromosomes remain unidentified. In conclusion, these observations illustrate the need for exercising caution when assigning the role of sex determination to long, heteromorphic-appearing pairs, even though a difference in length is considered a major criterion for distinguishing the X and Y chromosomes. (AEC Postdoctoral Fellow, 1951-53.)

YOON, CHAI H., The Ohio State University, Columbus, Ohio. A decrease in variability in hybrid generations of the time of vaginal opening in the house mouse. — The mean time of vaginal opening of three long inbred strains of mice, NB, C57BL/10, and BALB/c were found to be 24.803, 34.939, and 21.054 days, respectively, after the removal of seasonal effects by fitting data with a sine curve. The differences between means of these three strains are statistically significant. Crosses were made between NB and C57BL/10, and between BALB/c and C57BL/10. The means of the various generations of these crosses showed that the gene effects are additive on the natural scale when the seasonal effects are removed by a sine curve. However, the magnitude of the non-heritable variance of a generation was found to be associated with its degree of heterozygosity. It was found that the greater the increase of heterozygosity in a generation the smaller became its non-heritable variance.

ZALOKAR, M., National Institutes of Health, Bethesda, Md. Maternal effect in the inheritance of a modifier of the Bar mutant in Drosophila melanogaster. — The strain C1B/+ from Zurich [Zu] produced larger reniforme eyes with higher facet number than the strain from Geneva [Ge]. This difference was found to be due to a modifier gene (two genes ?) which was not sex-linked. The alleles of this modifier (s) were crossed into the Bar and wild-type and the facet counts gave following averages: B/B [Zu] females: 128, males: 140; B/B [Ge] females: 61, males: 83; B/+ [Zu] females: 588; B/+ [Ge] females: 205. The modifier had no effect on the facet number of wild eyes. Crosses between strains from Zurich and from Geneva showed a maternal effect on the facet number of the offspring which was most prominent in the heterozygous B/+ (reniform) eyes. Facet counts of different crosses gave: Bar [Zu] female x wild [Ge] male: 485; Bar [Ge] female x wild [Zu] male: 207; wild [Zu] female x Bar [Ge] male: 410; wild [Ge] female x Bar [Zu] male: 268. These results suggest that the modifier (s) was responsible for the production of a substance which increased the number of facets and that this substance was transmitted through the egg cytoplasm, influencing thus the facet number of the offspring.

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CORRIGENDA

Page 293, lines 3 and 4, (in equations) for "$2x_{11} + x_{12}$ etc." read "$2x_{11.} + x_{12.}$, etc."

Page 293, line 9, for "$\Sigma\Sigma\Sigma(X_{ijk} - \Sigma_{ij.})^2$" read "$\Sigma\Sigma\Sigma(X_{ijk} - X_{ij.})^2$."

Page 154, line 17 for "*M-5/+; +/+; Payne Dfd ca/1(3)F*" read "*M-5/M-5; +/+; Payne Dfd ca/1(3)F*".

Page 166, line 34 (in literature cited) insert WRIGHT, S., 1948 On the roles of directed and random changes in gene frequency in the genetics of populations. Evolution **2**: 279–294.

Page 233, table 3, (total of column 3 headed " Doubles " for " 25 " read " 24 ".

Page 426, equation (8), for "$\overline{A}_D$" read "A_D".

Page 435, footnote 6, for " (tables 2 and 3) " read " (tables 1 and 2) ".

Page 436, line 21, for "$n = 0$" read "$m = 0$".

Page 436, line 25, for "$n_o p_o^2 g^2$" read "$n_o p_o g^2$" and for "$n = 0$" read "$m = 0$".

Page 443, under Fisher, R. A. 1918, for " 62 " read " 52 ".

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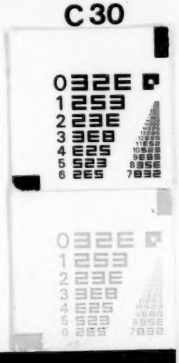
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